VOLUME 97 NUMBER 5

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

November, 1955

The Nature of Direct Bilirubin. BARTON CHILDS 333

Some Observations on the Hypophyseal Portal System in the Living Mouse. W. CURTIS WORTHINGTON, JR. 343

The Function of the Pituitary Stalk. (A Herter Lecture for 1955). G. W. HARRIS 358

Book Reviews 376

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

COPYRIGHT, 1955, BY THE JOHNS HOPKINS PRESS

BULLETIN OF

THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, E. K. MARSHALL, JR.

ASSOCIATE MANAGING EDITOR, VERNON B. MOUNTCASTLE

EDITORIAL BOARD

E. Cowles Andrus **Charles R. Austrian** **Henry T. Bahnson**
Kenneth C. Blanchard **John Eager Howard**
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Correspondence concerning business matters and all remittances should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

Subscription price. In the United States and its possession $8.00 per year. In Canada $8.25 per year; in foreign countries $8.50 per year. Single numbers, $1.00.

New subscriptions and renewals are entered to begin with the first issue of the current year. Subscribers in the United States are hereby notified that missing numbers cannot be replaced unless notification of loss is received in this office within 1 month following publication of the missing number; foreign subscribers 6 months.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2.

Manuscripts. Manuscripts should be typewritten, one side of page only, and double-spaced. Following the title should appear the author's name, department, and the university and/or hospital he represents. A list of references (bibliography) in numerical sequence should appear at the end of article. Preferred style for references is as follows: Author's last name, initials. Title of book or article, with first word and proper names capitalized only. Name of journal (abbreviate), date of publication, volume number: page number. The Bulletin is not responsible for loss of a manuscript in mail. Author should retain a duplicate copy.

Illustrations and Tables. Legends are required for Figures and descriptive headings for Tables. Use Arabic numerals to number Figures (Fig. 1); use Roman numerals to number Plates and Tables (Plate I). It should be indicated exactly where Figures and Tables are to appear in text; Plates all appear at end of article. All Figures should be drafted in black India ink for 50% reduction to 4½ inch width. Glossy photographs must be supplied for all Plates. Original illustrations will be returned to author with galley proofs.

Reprints. Twenty-five reprints, without covers, are furnished gratis for each article. Additional reprints should be ordered when galley proofs are returned.

THE JOHNS HOPKINS PRESS

BALTIMORE-18, MARYLAND, U. S. A.

Entered as second class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

THE NATURE OF DIRECT BILIRUBIN

BARTON CHILDS*

Harriet Lane Home, The Johns Hopkins Hospital, and the Department of Pediatrics, The Johns Hopkins University School of Medicine

Received for publication May 17, 1955

One of the most widely used and most dependable clinical tests now employed in medicine is the Van den Bergh test. It measures the total amount of bilirubin in an icteric serum, and indicates that proportion which has passed through the cells of the liver (direct bilirubin) and that which has not (indirect bilirubin). It is not known why the direct pigment reacts with the diazo reagent in the Van den Bergh test in the absence of alcohol, while the indirect does not. Neither is there precise knowledge of the nature of the metabolism of the pigment by the liver responsible for this difference.

There have been, however, many studies characterizing differences between direct and indirect bilirubin. Many of these have been concerned with the binding of the pigment by serum proteins. It was Van den Bergh's contention that direct bilirubin has an affinity for denatured protein, while the indirect remains free (1). Subsequent reports of work in which the Tiselius method of electrophoresis was used have indicated that both direct and indirect pigments migrate with the serum albumin (2, 3, 4). One study, however, found bilirubin bound to both albumin and the alpha globulin fractions (5). When separate protein fractions are dialysed against known amounts of commercial bilirubin the pigment is shown to be bound mol for mol with both albumin and alpha globulin, but not with beta and gamma globulins (6, 7, 8). Fractionation methods have also been used. Cohn used methyl alcohol and found bilirubin with both albumin and alpha globulin (9). Others, using ammonium sulfate, showed that the indirect precipitated with the globulins, while the direct precipitated with the albumin fraction (8).

An important differentiating feature is the observation of Najjar that indirect bilirubin can be extracted from serum by ether after adjustment of the serum to pH 5, after which the bilirubin can be crystallized (10). Only very small amounts of direct bilirubin are extracted under these circumstances, and crystallization is at best difficult and usually impossible.

Finally, Cole, Lathe, and Billing have separated direct and indirect pigments chromatographically, using a kieselguhr column (11).

These studies have all shown differences in the behavior of the direct and indirect bilirubin, but none has defined the chemical or physical nature of the difference. It has been indicated by Yamoka that the direct reacting pigment

* John and Mary Markle Scholar in Medicine.

may be an ester (12). Najjar has suggested that direct bilirubin might be a metal-bilirubin complex. He found that in the presence of divalent metal ions at high pH the direct pigment was readily oxidized to biliverdin (13). This reaction was prevented by the addition of versene (ethylene-diamine-tetra-acetic-acid). In addition, versene or other chelating agents were found to alter the Van den Bergh reaction of direct bilirubin causing it to react only following the addition of alcohol. These observations suggested that the direct bilirubin might be in the form of a metal complex.

The purpose of this paper is to present some observations on the electrophoretic mobility of bilirubin using a filter paper technique, and a further testing of the hypothesis that direct bilirubin exists as a metal-bilirubin complex.

METHODS

The sera used were obtained at the Johns Hopkins Hospital and Sinai Hospital, from patients suffering from jaundice due to the following conditions:—Congenital atresia of the bile ducts; obstructive jaundice due to hepatitis, cholangitis, or other causes; erythroblastosis fetalis; physiologic jaundice of the newborn; Sickle cell disease; and familial, non-hemolytic jaundice. Serum was also obtained from a dog whose bile duct had been ligated and divided (kindness of Dr. James Cantrell).

For the electrophoresis runs the apparatus used was Sorvall LKB Paper Electrophoresis. The paper was Munktell no. 20. Buffers used were; Barbital 0.06 m., pH 8.6; phosphate 0.2 m., pH 7.5; phosphate 0.2 m., pH 6.5; phosphate 0.2 m., pH 6.0; acetate 0.2., pH 5.5; acetate 0.2 m., pH 5.0. The papers were soaked in the appropriate buffer, and placed in the apparatus. At a point marked with pencil 0.02 or 0.04 cc. of serum was delivered from a micropipette. The current was set at 2 milliamperes per strip and the run completed in 7 to 18 hours depending upon the pH of the buffer used. At high pH values good separation could be accomplished in 7 hours, but at pH 6.5 or below, separation was best if the run was allowed to continue overnight for 17–18 hours. After the run the paper was dried at 100° in the oven. The bilirubin spot or spots, which were bright yellow or green, were then ringed with pencil and the papers stained with AzO-carmine B (14). The excess dye was removed by washing and the papers again dried in the oven.

Quantitative estimations of direct and indirect pigments were made by the Van den Bergh test as modified by Malloy and Evelyn (15).

RESULTS

Experiments were carried out on 20 different sera, 12 containing predominantly direct bilirubin, 8 containing predominantly indirect bilirubin. Nine of the sera were subjected to electrophoresis runs in all the buffers mentioned varying in pH from 8.6 to 5.0. Results were quite uniform and comparison between a direct serum and an indirect is shown in Figure 1. It will be seen that at pH 8.6 both direct and indirect bilirubin migrate with the albumin fraction, and that at all pH values the direct remains with this protein fraction. On the other hand, at pH values of 6.5 and below progressively more of the indirect pigment dissociates from the albumin remaining at or near the starting point, until at pH 5.0 none of the bilirubin has migrated. This appears to be due, not to the

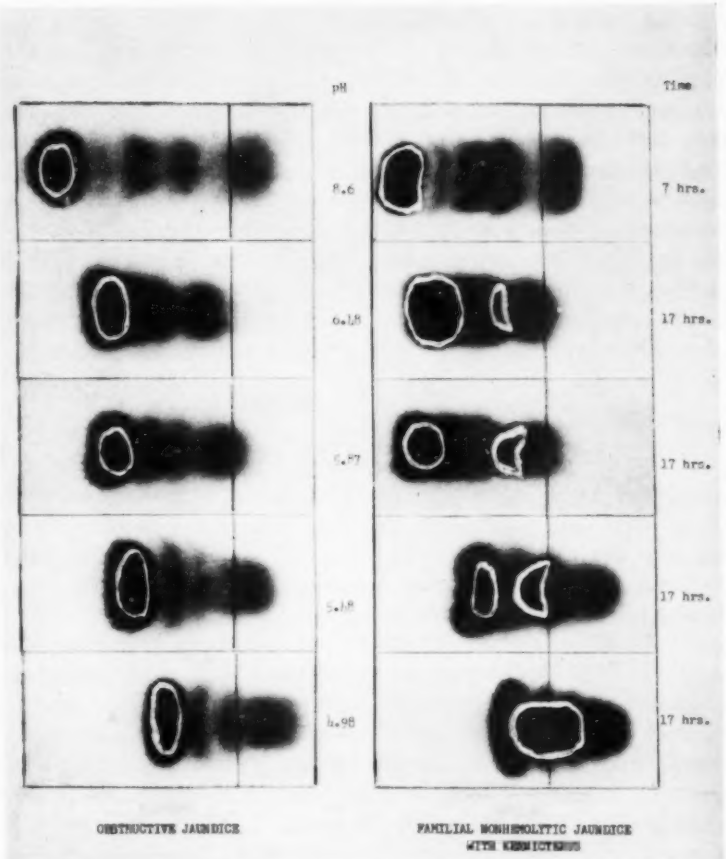


FIG. 1. Paper electrophoresis of sera containing direct and indirect bilirubin. The bilirubin spots on the paper have been outlined with white ink.

attachment of direct and indirect bilirubin to different protein fractions, but to the easy dissociability of indirect bilirubin at low pH as opposed to the tight combination with the direct albumin. Thus at low pH, the indirect bilirubin does not migrate at all but comes out of solution altogether. This is not surprising since it is this property which enables one to crystallize indirect bilirubin after extraction with ether (10).

In order to test whether or not the bilirubin might have changed its properties with reference to the Van den Bergh test through exposure to high and low pH, the following procedure was carried out. The electrophoresis run was carried out as usual, the papers were dried and then cut longitudinally, cutting

through the center of the bilirubin spot. One half was then dyed with Azo-carmine to visualize the relation of the bilirubin to the protein fractions, and the bilirubin spot on the other half was dipped into acidified diazo reagent, and subsequently, if no coupling occurred, into methyl alcohol. By this means it could be shown that the pigment retained its original properties in relation to this test regardless of pH or attachment to or detachment from the albumin. It was of interest that during the longer runs some of the direct bilirubin was oxidized to biliverdin so that the bilirubin spot became a bright, yellow green, whereas the indirect retained its yellow color. When sera containing both direct and indirect bilirubin in approximately equal quantity, were run at pH 5.0 two spots would appear. One remained at the starting point and consisted of indirect bilirubin, the other migrated with the albumin and consisted of direct bilirubin.

Effects of versene:

In order to test the effects of a chelating agent on direct bilirubin, 12 sera containing 70 per cent or more of direct bilirubin were dialysed against solutions of versene (tetra sodium salt of ethylene diamine tetra acetic acid). The versene solution used was 0.25 molar, adjusted with HCl to pH 9.5–10.0. The sera were dialysed in 5 or 10 cc. of the versene solution overnight at 4° C. As a control, the same sera were dialysed under the same conditions against 0.25 m. sodium acetate at pH 9.5–10.0. Control runs of these sera at pH 5.0, before dialysis, showed a single bilirubin spot superimposed upon the albumin fraction. When the dialysed sera were run at pH 5.0 it was found that those treated with versene showed two spots on the paper, one migrating with albumin, the other remaining at the starting point. Those dialysed against sodium acetate behaved exactly as the undialysed serum. This suggested that some of the direct bilirubin had been converted to the indirect form. In addition it was seen that the spot at the starting point retained its original yellow color, whereas that which had moved with the albumin had taken on the greenish hue which indicated oxidation to biliverdin.

To test by means of the diazo reaction for conversion by versene of direct to indirect pigment, separate papers were run which were cut longitudinally as before and one half the bilirubin spots were treated with diazo solution, and if no color developed, methyl alcohol was added. In every case the bilirubin which remained at the starting point had been converted to indirect while that which had moved remained direct.

The only known effect of versene under these conditions is to chelate metals. It seemed desirable to test the possibility that this compound might act in some other way, by using a versene already tightly bound to a metal which could not easily be displaced. Accordingly, sera containing direct bilirubin were dialysed against lead-versenate and then subjected to electrophoresis. Figure 2 shows

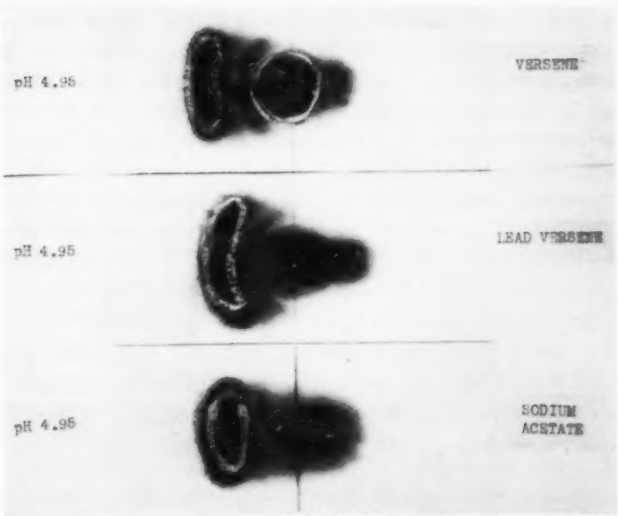


FIG. 2. Paper electrophoresis of sera containing direct bilirubin after dialysis against versene, lead versenate, and sodium acetate. The bilirubin spots have been outlined with white ink.

a comparison between runs on three samples of the same serum, one dialysed against sodium acetate, one dialysed against versene, and one dialysed against lead versenate. The results show that while versene converted a large proportion of the direct bilirubin to indirect, the lead versene had very little effect, and indeed the pattern looked as if no versene had been added at all.

Indirect bilirubin, being more readily dissociable from serum protein than direct, will pass through the wall of a cellophane bag. Dialysis could therefore be a test for the presence of indirect pigment. Direct sera were therefore dialysed overnight and in the cold against 5 cc. of 0.25 molar versene and as a control 0.25 molar sodium acetate, both at pH 9.5–10.0. The absorption spectrum of the color in the dialysate was then read in the Beckman spectrophotometer to ascertain that the yellow color was indeed bilirubin. In each case, the amount of bilirubin was several times greater in the versene dialysate than in the sodium acetate dialysate, the latter being in most cases nearly colorless. Dialysis against Pb versene showed the escape of an amount of bilirubin slightly greater than that obtained in dialysis against sodium acetate, but considerably less than when versene was used. A representative graph is shown in Figure 3. When indirect sera were dialysed against versene and sodium acetate, there was no significant difference in the amounts of bilirubin which escaped.

To obtain further evidence of conversion of direct to indirect bilirubin, quantitative measurements of the two fractions were compared before and

after the addition of versene. To direct sera were added in equal parts, solutions of versene varying in concentration from 0.05 molar to 1 molar, after which the sera were brought to pH 10 by addition of HCl. It was found that 1 molar versene sometimes precipitated the proteins, while 0.1 molar versene or less, failed to produce as much conversion as more concentrated solutions. The most workable solution was, therefore, found to be 0.5 molar versene, the final concentration of versene being 0.25 molar. This mixture was then incubated overnight at 37°. Because appreciable amounts of versene appeared to interfere with reproducible quantitative results in the Van den Bergh test, the sera were dialysed in large quantities of distilled water before carrying out the test. This resulted in considerable loss of bilirubin which passed through the wall of the bag. This loss was indirect bilirubin which had been converted from direct, since direct pigment is not dialysable. Indeed quantitative comparison of untreated serum before and after dialysis showed no change in the direct fraction,

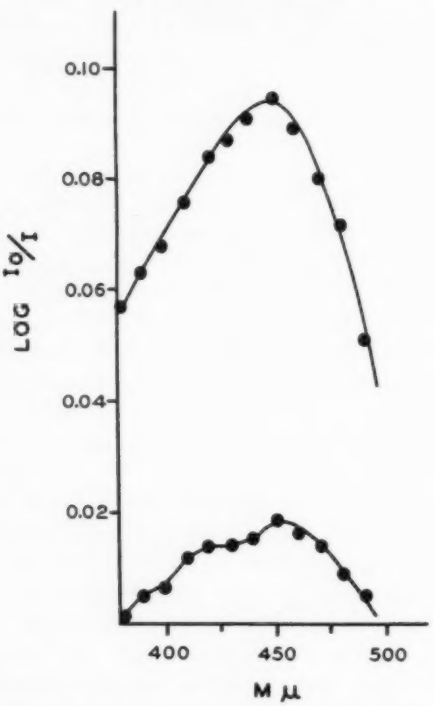


FIG. 3. Comparison of dialysis of serum containing direct bilirubin against versene, lead versene, and sodium acetate. Upper curve; difference in absorption between dialysis against versene and sodium acetate. Lower curve; difference between lead versene and sodium acetate.

Bilirubin mg. Per Cent

Patient	Versene Conc.	Untreated Serum			Serum + Versene			% Conversion
		Direct	Total	% Direct	Direct	Total	% Direct	
	m							
G	0.025	6.2	7.4	83.78	5.5	6.2	88.71	11.30
HU	0.025	7.8	10.2	76.47	6.2	10.0	62.00	20.51
K	0.25	7.6	9.6	79.17	3.0	3.6	83.33	60.53
D	0.25	3.2	4.6	69.56	1.6	2.2	72.72	50.00
L	0.5	7.5	8.8	84.58	2.18	4.02	54.23	70.88
L	0.25				2.12	4.44	47.74	71.59
L	0.05				4.36	7.72	56.48	41.56
REA	0.5	12.2	15.7	77.70	4.6	6.7	68.66	62.20
REE	0.5	6.48	9.00	72.00	2.4	3.5	68.97	62.96

FIG. 4. Quantitative estimation by means of The Van den Bergh diazo reaction of conversion of direct to indirect bilirubin following treatment with versene.

but losses in the indirect. The amount of direct converted to indirect was therefore considered equivalent to the difference between the values of the direct before and after incubation and dialysis. The results of quantitative tests with 9 sera are set out in Figure 4. The conversion in the first two sera was relatively

FIG. 5. Bilirubin crystals obtained from a serum treated with versene. The original serum contained a total bilirubin of 8.8 mg. % which 7.5 mg. % gave the direct reaction.

little. In these cases, however, the final concentration of versene used was only 0.025 molar. The effect of more versene on the same serum will be seen when comparing the two runs on serum L, in which conversion was nearly doubled when the final versene concentration was raised to 0.25 molar. Thus with exposure to adequate amounts of versene it is possible to convert as much as 70 per cent of direct bilirubin to indirect.

As a final step it was necessary to show that the converted bilirubin could be readily crystallized from the serum treated with versene. Accordingly, suitable sera containing almost all direct bilirubin, and very little indirect, were treated with sufficient versene to make a 1.0 molar solution and were incubated overnight at 37°. These were then treated by the method of Najjar and crystals were obtained which were identical to those which can be easily obtained from any serum containing naturally indirect bilirubin, as may be seen in Figure 5. Crystallization could not be accomplished with original untreated sera.

These crystals were then redissolved by raising the pH with sodium hydroxide. When this was done and bivalent metal ions added, the solution became blue green indicating oxidation of the bilirubin to biliverdin suggesting that the pigment had formed a metal complex that was readily oxidizable. This reaction could be prevented by the addition of versene.

DISCUSSION

It is known that there are certain differences in the way in which the body handles direct and indirect bilirubin. For example the kidney will not excrete the indirect, but will readily excrete the direct form. In cases of regurgitation jaundice the skin and tissues show a greenish color not seen in retention jaundice, suggesting the presence of biliverdin, an oxidation product of bilirubin. This pigment has been measured and found to be elevated in some cases (16). It is important if one would understand these clinical differences to attempt to characterize the metabolism of the pigment in the body.

It is clear that there are no physical or chemical differences between indirect bilirubin crystallized from serum of patients with retention jaundice and that commercially available, which has been isolated from bile (8). This suggests that bilirubin which has been metabolized in the liver is not irreversibly altered in that process. On the other hand there is evidence of notable differences between direct and indirect bilirubin as they exist in serum. The direct form is easily oxidized, and is very tightly bound to albumin, defying efforts to extract it with ether at low pH or to crystallize it, and resisting dialysis. These characteristics, however, have now been shown to be in large part altered by the addition of versene, a metal binding agent, after which the pigment then behaves according to all criteria, including the Van den Bergh test, like the indirect form. So far as is known, the only effect of versene, under the conditions used, is to form complexes with metals, and its ability to do so is of a very high

order. The fact that versene has apparently converted direct to indirect bilirubin is evidence that the direct form is a metal-bilirubin complex. It is worth noting that when the versene was already tightly bound with lead, the conversion was markedly impaired. Thus these observations appear to indicate that direct bilirubin which finds its way into the blood in obstructive jaundice exists in tight combination with the serum albumin fraction as a bilirubin-metal-protein complex. An interesting example of such a ternary complex has been given by Klotz (17). In this work it was shown that an uncharged azopyridine dye (Pyridine-2-azo-p-dimethyl-aniline) could be complexed with albumin and pepsin by the addition of metal ions. Without the metal ions, the dye shows no affinity for the proteins. In the case of the bilirubin-metal-albumin complex, the nature of the metal involved, and the circumstances of its union with the pigment require further investigation.

SUMMARY

Observations have been presented indicating that direct bilirubin exists in serum as a bilirubin-metal-albumin complex. The addition of versene to sera containing direct bilirubin can bring about the conversion of a considerable proportion of the pigment from direct to indirect as measured by several different criteria. These include: the Van den Bergh test, ease of oxidation of bilirubin to biliverdin, dialysis through a cellophane bag, migration in paper electrophoresis, extraction with ether at pH 5, and crystallization.

BIBLIOGRAPHY

1. Van den Bergh, A. A. H.: *Die Gallenfarbstoffe im Blutle, J. A. Barth, Leipzig,* 1918.
2. Benhold, H.: Über die Vehikelfunktion der Serumeiweisskörper. Ergebnisse der Inn. Med. und Kinderheilk. 1932, **42:** 336.
3. Pedersen, K. O. and Waldenstrom, J.: Studien über das Bilirubin in Blut und Galle mit Hilfe von Elecktrophorese und Vetrazentrifugierung. Hoppe-Seiler Z. Physiol. Chem. 1937, **245:** 152.
4. Coolidge, T. B.: Chemistry of the Van den Bergh reaction. J. Biol. Chem. 1940, **132:** 119.
5. Gray, C. H. and Kekwick, R. A.: Bilirubin serum protein complexes, and the Van den Bergh reaction. Nature 1948, **161:** 274.
6. Martin, N. H.: Preparation and properties of serum and plasma proteins, XXI: interactions with bilirubin. J. Am. Chem. Soc. 1949, **71:** 1230.
7. Barac, G.: Interactions of bilirubin with plasma proteins and the state of bilirubin in normal plasma. Arch. internat. physiol. (Paris), 1953, **61:** 129.
8. Najjar, V. A. and Childs, B.: The crystallization and properties of serum bilirubin. J. Biol. Chem. 1953, **204:** 359.
9. Cohn, E. J.: The chemical specificity of the interaction of diverse human plasma proteins. Blood 1948, **3:** 471.
10. Najjar, V. A.: Crystallization of direct and indirect bilirubin. Fed. Proc. 1951, **10:** 227.

11. Cole, P. G., Lathe, G. H. and Billing, B. H.: Separation of the bile pigments of serum, bile and urine. Biochem. J. 1954, **57:** 514.
12. Yamakoa, K. and Kosaka, K.: Studies of bile pigments. Proc. Japan Acad. 1951, **27:** 715.
13. Najjar, V. A.: The metabolism of bilirubin. Pediatrics 1952, **10:** 1.
14. Pluckthun, H. and Gotting, H.: Beitrag zur Methode der Elecktrophoretischen untersuchung der Serumeiweisskörper auf Filtriepapier. Klin. Woch. 1951, **29:** 415.
15. Malloy, H. T. and Evelyn, K. A.: The determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem. 1937, **119:** 481.
16. Watson, C. J.: The bile pigments. New England J. Med. 1942, **227:** 665.
17. Klotz, I. M.: Thermodynamic and molecular properties of some metal protein complexes. In *Mechanism of Enzyme Action*, McElroy, W. D., and Glass, H. B., eds. Johns Hopkins Press, Baltimore, 1954, p. 257.

SOME OBSERVATIONS ON THE HYPOPHYSEAL PORTAL SYSTEM IN THE LIVING MOUSE

W. CURTIS WORTHINGTON, JR.

Department of Anatomy, The Johns Hopkins University School of Medicine

Received for publication May 31, 1955

Since the original description of the hypophyseal portal system in man by Popa and Fielding (1), a large body of evidence from a variety of animals has been accumulated indicating that the flow in these vessels is from the median eminence toward the pars distalis, contrary to the opinion of the original investigators. This was first deduced on morphologic grounds by Wislocki and King (2) and has since been confirmed on the same grounds by many investigators (3–10). Direct evidence in the form of observations of the hypophyseal portal vessels in the living animal has been supplied by Houssay, Biasotti, and Sammartino (11), who described a portal system in the toad and found that blood flow was toward the pars distalis. This has been confirmed in the bullfrog by Green (12), and in the rat by Green and Harris (13).

The hypothesis has been suggested and repeatedly restated (6, 7, 12, 14–18 and others), that the hypophyseal portal vessels represent a functional link between the nervous system and the pars distalis. The lack of convincing evidence for the presence of secreto-motor fibers in the pars distalis, which has been thoroughly discussed by Green (19–21), has emphasized this concept. A great deal of experimental work has been directed toward testing the hypothesis that a humoral substance released into the portal system in the median eminence and stalk is responsible for the control of the release of some of the hormones of the pars distalis. Vasomotor activity within the portal system has received some consideration as a possible regulating factor. The whole problem has been reviewed by Green and Harris (7, 8, 18, 21–23) and DeGroot (24).

In view of the highly important function tentatively ascribed to this system, it seemed desirable to obtain as detailed information as possible about the blood flow in the various components of the system in the mammal, not only under conditions as nearly normal as possible, but also under controlled experimental conditions. The vessels of the stalk have been studied in the living rat by Green and Harris (13), who described certain aspects of the circulation, particularly the direction of blood flow in the portal veins. However, they did not consider vasomotor changes in the finer vessels in the system.

The present report deals with observations in more than fifty living mice. This study was undertaken in order to describe, in those hypophyseal vessels which may be brought under direct observation without microscopically visible evidence of trauma to the vessels, the vasomotor activity, the comparative

rates and direction of flow, and intermittence or lack of it in the component small vessels of the system. It was found after examining the first few successful preparations that some supplementary anatomic studies would have to be made in the living animal before a maximum of information could be obtained from physiologic studies.

MATERIALS AND METHODS

The animals used in these experiments were albino mice, obtained from Carworth Farms (strain CF1). Both males and females were studied, but the majority were males weighing from 13 to 25 grams.

The exposure of the ventral surface of the stalk, its attachment at the median eminence, and a large center portion of the pars distalis was accomplished in the following manner: Under intra-peritoneal pentobarbital sodium anesthesia (.65 mg/10g. body wt., initial dosage), the animal is restrained on its back on a cork board by thin tapes about the extremities, and a thin strand of silk thread around the upper incisors. Hair is clipped from the region of the neck and lower jaw. A midline incision is made from the interramal vibrissae to the sternal notch, and the subcutaneous tissue is divided with scissors. The submaxillary glands are separated by gentle blunt dissection with a moist cotton pledget, and the fascia is removed from the muscles overlying the trachea. At this point, very fine insect pins are passed through the skin margins and secured to the cork board for retraction. The sternohyoid muscles are incised, divided and removed. The trachea is gently separated from the esophagus, divided completely with scissors, retracted posteriorly, and supported at the sternal notch with a fine insect pin through the small amount of adherent fascia on the dorsal surface.

The esophagus is then retracted laterally, and the tracheal stump anteriorly, by curved insect pins secured to the cork board by thumb tacks and threads. A third retractor is placed at the lateral margin of the retracted tracheal stump on the side opposite the retracted esophagus, which may be divided if necessary. The posterior pharyngeal wall is then elevated with a fine cotton pledget, with further retraction if necessary. The attachment of the longus capitis muscle just posterior to the basisphenoid synchondrosis is scraped away with a dental chisel, and the area is thoroughly irrigated with mammalian Ringer's solution.

At this point in the procedure the bone is burred away from a point about one mm. behind the basisphenoid synchondrosis to a point just posterior to the transverse sinus of the basisphenoid. By careful drilling with the burr the outer table of bone can be removed. A small amount of bone wax is used to tip the dental burr to prevent bleeding from bone, but it is not necessary to pack the transverse sinus with wax unless wide anterior exposure is desired. When the outer table is completely removed, the inner table is removed in the following

manner: A small flat dental chisel is insinuated through the synchondrosis. This is passed at an angle for a very short distance anteriorly and the inner plate of bone is snapped out by upward pressure. The bone usually does not come out in one piece, and remaining fragments about the edges are removed in a similar manner. The area is immediately irrigated with mammalian Ringer's solution. This is taken up with a very fine suction tip, taking care that this tip does not touch the underlying soft tissue. Unfortunately, some oozing of blood from the bony margins of the defect is encountered with some frequency. If it occurs the animal is left alone for a few minutes and the clot is removed with jewelers forceps by grasping it at the margin. The area is once again irrigated and the excess fluid taken up by suction. Frequently the dura mater was not disturbed, many observations being made through the transparent membrane. All steps in the procedure from exposure of the inner table of bone are carried out under a dissecting microscope.

If exposure of a larger area of the pars distalis is desired, the bony defect may be carried further posteriorly. It seemed desirable however, in order to minimize trauma, to expose as small an area as the demands of the study would allow. In this work principal attention was directed toward the primary capillary "plexus", its arterial supply, and the portal veins themselves, and only a small area of the ventral surface of the pars distalis was routinely exposed.

Illumination was supplied by a microscope lamp directed onto the surface of the area under study. Two heat-absorbing glass filters were placed in the beam, and various color filters were used when they were found helpful. Microscopes used have been a Bausch and Lomb binocular dissecting microscope ($\times 7$ objective and $\times 15$ oculars) and a standard American Optical Company compound microscope ($\times 10$ objectives and $\times 15$ oculars). Fogging of the surface of the compound objective was prevented by treating it with Laboratory Aresol obtained from the Fisher Scientific Company.

The epinephrine used in these experiments was in the form of epinephrine hydrochloride, Parke-Davis, and l-epinephrine bitartrate supplied by Winthrop-Stearns. All tests were made with both forms of the drug, in equivalent doses, in different animals. The Methacholine chloride used was in the form of Mecholyl chloride obtained from the Sharp & Dohme Division of Merck & Co. One drop (c. 0.05 ml.) of the desired dilution was applied to the exposed field and the excess immediately aspirated.

The criteria for normalcy of the vessels which have been brought under observation as far as local injury is concerned are those of Clark and Clark (25) as supplemented by Brooks, Dragstedt, Warner and Knisely (26). It is possible to maintain these preparations for several hours without microscopically visible evidence of local damage. Animals will live for many hours even when severe local damage has occurred.

OBSERVATIONS

The pattern formed by the arteries supplying the primary capillary system is highly variable. In general, the superior hypophyseal arteries have been observed to emerge from beneath the margins of the bony defect just posterior to the rostral limit of the system (Fig. 1). Two major variations in the arterial pattern are an arterial loop lying just anterior to the rostral limit of the system (Figs. 2, 3, 4, 5), and a large artery which passes along the lateral border of the stalk (Figs. 1, 3, 4, 5). The latter vessel may be absent; when this is the case, a vessel of similar diameter may be seen approachig the stalk at right angles at a lower level (Fig. 2). The arterial loop varies in its structure and shows changes in vasomotor activity. It may be fairly wide and of uniform diameter, representing arteries from the two sides in smooth continuity, forming an arc anterior to the median eminence with its concavity facing posteriorly (Fig. 2). The anastomosis, when it occurs, may be made by a much narrower vessel (Fig. 3). The arteries supplying the primary capillary system have varied widely in diameter. One of them may be quite large as it emerges from beneath the margins of the bony defect, tapering rapidly to a much narrower diameter as it approaches the primary capillary system (Fig. 3). Anastomoses between primary branches of the superior hypophyseal arteries have been observed.

Arterioles supplying the primary capillary system arise from branches of the superior hypophyseal arteries, and from the concavity of the arterial loop, when it occurs. The artery at the lateral border of the stalk supplies several branches to the stalk. There is frequently a long arteriole which passes almost the entire length of the stalk about halfway between the lateral border and the midline (Figs. 1, 2). It is sometimes paired, and in one case an H-shaped anastomosis between such a pair was observed just above the junction of the stalk with the gland. In some preparations this arteriole disappeared into the substance of the stalk. In others it terminated in a manner similar to others higher on the stalk. Some of the arterioles branch at the periphery of the system giving a T-shaped structure, each arm of the T passing for a short distance circumferentially before turning into the system (Figs. 1, 2).

From the arterioles very fine capillaries arise, but many if not all of these arterioles do not terminate by breaking up into these fine, rather irregularly distributed capillaries. They continue until they join wide capillaries which have certain attributes which distinguish them (Fig. 6). These wide capillaries occur in recognizable groups. The individual vessels anastomose with each other, many form loops anteriorly, and frequently have an undulatory configuration along their individual courses. In general, the orientation of these groups is longitudinal. Wide capillaries within a group are interconnected by fine capillaries, and capillary interconnections occur between groups (Fig. 6). These groups are frequently not visible in their entirety, and many capillaries are observed to emerge from the substance of the median eminence (Fig. 7).

The termination of the arterioles is frequently an abrupt one on a loop formed by the capillaries within a group at its proximal end (Fig. 8). Less frequently the arteriole gradually widens into a space which approaches the diameter of a small portal vein. From this space the wide capillaries of the group arise (Fig. 9). One other connection of these arterioles has been seen a few times. Fine twigs from an arteriole have been seen to pass directly to the proximal end of a small portal vein.

Capillaries converge at the distal end of a group frequently forming a collecting vessel, which joins one or more similar vessels to form a portal vein. A single group may form a small portal vein. A group may supply two portal veins by means of the bifurcation of a collecting vessel (Fig. 10). Anastomoses of large diameter have been observed between portal veins (Fig. 11). Portal veins and collecting vessels, as well as primary capillaries, have been observed to emerge from the median eminence (Fig. 12).

As has been described in previous studies on fixed tissues, the portal veins may arise at any level of the stalk or median eminence. They pass down the stalk to the pars distalis, where they branch and rebranch forming the sinusoids of the pars distalis.

This somewhat complex vascular system has shown some interesting activity. Blood flow in the primary capillaries, portal veins and anterior lobe sinusoids is readily visible under most circumstances. This is not true of the arterial vessels. The rate of blood flow in portal veins of approximately the same diameter may be different. Pulsations may sometimes be seen in the larger arteries, but not in arterioles or portal veins. The arterioles are capable of dilating widely, narrowing markedly, and under some circumstances of disappearing from the field completely. Groups of capillaries, under the influence of locally applied epinephrine, may disappear from the field as a unit, adjacent groups remaining open. The fine interconnecting capillaries within and between groups sometimes show a rapid intermittence, opening and closing many times in a minute. They may remain closed for much longer periods. Blood flow in the capillaries emerging from the median eminence may be in either direction and may change direction (Fig. 6).

Blood flow in a segment of the arterial loop lying anterior to the median eminence has been observed to slow down markedly with or without a change in direction (Figs. 2, 3). The narrow vessel which sometimes completes this loop is capable of closing completely. A vessel completing such a loop may not be apparent until a vasodilator is applied (Fig. 5). Reversals in flow and complete closure of arterio-arterial anastomoses between primary branches of the hypophyseal arteries on the same side have been observed.

Subcutaneous administration of epinephrine (2γ/kg.) is associated with marked acceleration of flow rates in portal veins. The flow is so rapid that it is no longer visible. Local application of epinephrine is concentrations as low as

1:5,000,000 is associated with narrowing of arterial vessels and diminution of flow rates in primary capillaries and portal veins. Application of Methacholine chloride 1:100,000 is associated with dilatation of arterioles and acceleration of flow rates in portal vessels.

Reversals in direction of flow have been observed in arterio-arterial anastomoses, in interconnecting capillaries within and between groups on the surface, and in capillaries emerging from the median eminence.

In this series of more than fifty mice blood flow in the collecting vessels and the portal veins themselves has always been toward the pars distalis.

DISCUSSION

The advantages and disadvantages of *in vivo* study in relation to vascular injections require some comment. The principal advantage is the observation of vasomotion and differences in rates and directions of flow in vessels of different structure, size and orientation. The fine ramifications of the arterial tree can, in many instances, be traced to their terminations quite readily in the living preparation. These terminations, it should be apparent, are frequently in no sense a break-up into capillaries, but a distinct and sometimes abrupt termination on vessels of entirely different structure. The groupings of wide capillaries are probably more apparent in the living preparation, for the number of open capillaries between groups is variable. Under some conditions, especially of local trauma, so many vessels may be open that the groupings are not apparent on casual examination.

The disadvantages are numerous. Many vessels cannot be brought into view. Vessels passing deeply into the substance of the median eminence or stalk cannot be traced to their origins or terminations. Only acute experiments have proved feasible up to this time. Most data must be recorded by means of notes and sketches, although motion pictures of the larger vessels have been taken. Effective magnifications greater than 150× are difficult to obtain because of lack of working space and inadequate illumination. Many of the effects of stress on anterior lobe function are well known, and these animals are under stress from the moment of the initial incision. The effects of anesthesia must also be taken into consideration when interpreting the results of these experiments. Despite all of these defects this method should not be without value in obtaining data which may aid in clarifying the role of the hypophyseal portal system in the control of anterior lobe secretion.

The gross anatomical findings in regard to the larger arteries supplying the primary capillary system in the mouse, and the notation of a large vascular loop anterior to the median eminence, agree with the injection studies of Morin and Bottner (27), and in general the ramifications of the major arteries agree with the description of Landsmeer (28) in the rat. Both of these papers describe the large artery passing down the lateral border of the stalk, Morin

and Bottner describing its entrance into the pars distalis, and Landsmeer giving a more detailed discussion of its passage through the pars distalis and its distribution to the posterior lobe. He observed no branches to the sinusoids of the pars distalis, and designated this vessel the posterior lobe artery. Small branches to the stalk from this vessel have been observed in the living preparation, but none which passed to the pars distalis. However, its entire course cannot be traced in the preparation used in this study. In view of the fineness and contractility of some of the arterioles supplying vessels in the upper part of the system, the question of a direct arterial blood supply from the posterior lobe artery might well be left open until fine arterial vessels can be searched for in a living preparation. Such a preparation should not be technically impossible.

Observations in the rat (and several other forms) by Green and Harris (12), in the rat and mouse by Morin and Bottner (27), and in the rat by Landsmeer (28), indicate the presence of loops of sinusoidal capillaries penetrating the median eminence. These have been tentatively identified in the living mouse with those wide capillaries emerging from the median eminence in which reversals of blood flow have been seen. These vessels anastomose with wide capillaries visible on the surface.

The precise connections of some of the narrow, more irregularly distributed capillaries are still somewhat obscure, but many of them join the groups of wide capillaries. Whether or not the occurrence of terminal arteriolar twigs passing directly to portal veins is a purely incidental finding in a few animals, or a widespread and constant occurrence but obscured by some change in physiologic state not adequately controlled, remains to be determined.

It would appear from this study, and from previous injection studies by others, that the primary capillaries represent a system of small blood vessels in orderly sequence, showing differences in structure and perhaps having different functional capabilities.

Several implications may be derived from these studies. The functional data presented in the previous section suggest a very delicate mechanism controlling the rates at which blood from the median eminence and stalk, and perhaps from the general circulation, can reach the pars distalis. It has been suggested that humoral substances affecting or controlling anterior lobe secretion are formed in the median eminence and transported to the pars distalis by the portal system. The rates at which such substances reach the pars distalis would be affected by any vasomotor activity which changed the rates of flow in the portal vessels, unless some precise and inflexible reciprocal relationship between flow rates and the rates of formation and release of such substances exists.

In addition, if all or almost all of the blood which reaches the secretory cells of the pars distalis is derived from the portal system, a concept which the existing evidence seems to favor, then the rates at which substances in the

peripheral blood (especially the hormones of the target organs) reach the pars distalis would come under control of the portal system.

If specific areas in the median eminence or stalk are secreting, or specific groups of capillaries are receiving, different substances which are delivered to the pars distalis, control of the rates of flow through such specific areas under different physiologic conditions would have an effect on the rates of arrival of these substances in the pars distalis, and the relative proportions of such substances which arrive there. Thus flow rates in a given group could determine the capacity of the region to deliver its substance at a maximal rate. This could be extended to include the rates at which target organ hormones could be brought to the sites of production or release of these hypothetical substances.

Any experiments involving the direct application of drugs to the pituitary should be interpreted with caution unless direct microscopic examination of the vessels of the stalk, median eminence and pars distalis can be made for evidence of vascular damage and vasomotor changes, and any such changes should be taken into consideration when interpreting the results. Tension on the capsule of the gland can result in thrombosis of portal veins and anterior lobe sinusoids. If the membranes are removed and the area touched at all, intravascular agglutination, plugging of vessels, the sticking of white cells to vessel walls, and petechial hemorrhages occur almost immediately. Local application of epinephrine or Methacholine chloride bring about rapid changes in the diameter of arterial vessels and the rates of flow in portal veins.

Certain aspects of the hypophyseal portal circulation as observed in the living animal may have some bearing on the pathologic physiology of the pars distalis. The arterioles supplylng the system are very narrow and highly contractile. Embolization and/or spasm of these vessels might result in varying degrees of anoxemia of the pars distalis (barring a direct arterial blood supply not involved in the process), and partial deprivation of target organ hormones and other substances in the peripheral blood as well as the hypothetical substances of the median eminence. Severe degrees of intravascular hemagglutination such as are seen in burns and traumatic shock (and many other conditions) could well contribute to the reduction of the rate of blood flow through the portal system, with a resultant deprivation of the several substances indicated above. Trauma to or pressure on the veins draining the pars distalis results in engorgement and cessation of flow in the portal veins and anterior lobe sinusoids. Any process which caused pressure on these veins might well contribute to thrombosis of the portal system.

SUMMARY AND CONCLUSIONS

A method is described for exposing and studying in the living mouse the primary capillary vessels of the hypophyseal portal system, their arterial supply, the portal veins, and a portion of the ventral surface of the pars distalis.

Groups of wide anastomosing capillaries supplied by fine, highly contractile arterioles were observed. Fine capillaries interconnected the wider vessels within a group and capillaries occurred between groups. Intermittence of flow was observed in the vessels of the primary capillary system. Fine arteriolar twigs passing directly to the proximal ends of portal veins were seen in a few cases. Wide anastomoses occurred between portal veins.

Parenterally administered epinephrine was associated with increase in flow rates in primary capillaries and portal veins. Locally applied epinephrine was associated with constriction of arteries and arterioles and decrease in portal veins flow rates, while locally applied Methacholine chloride was associated with dilatation of arterioles and acceleration of portal vein flow rates. Both portal veins and primary capillaries were seen emerging from the median eminence and stalk. Blood flow in these capillaries was observed to change directions. In each of the more than fifty mice examined blood flow in the portal veins was toward the pars distalis.

These data suggest a very delicate mechanism controlling the rates of blood flow through the portal vessels and possibly through specific areas of the median eminence and stalk. By controlling the rate at which substances in the portal blood may be introduced into the humoral environment of the secretory cells of the gland, this system may have a profound influence on anterior lobe function.

REFERENCES

1. Popa, G. T. and Fielding, U.: A portal circulation from the pituitary to the hypothalamic region. J. Anat., 1930, **65:** 88–91.
2. Wislocki, G. B. and King, L. S.: The permeability of the hypophysis and hypothalamus to vital dyes with a study of the hypophyseal vascular supply. Am. J. Anat., 1936, **58:** 421–472.
3. Wislocki, G. B.: The vascular supply to the hypophysis cerebri of the cat. Anat. Rec., 1937, **69:** 361–387.
4. Wislocki, G. B.: Further observations on the hypophysis cerebri of the rhesus monkey. Anat. Rec., 1938, **72:** 137–150.
5. Morato, M. J. X.: The blood supply of the hypophysis. Anat. Rec., 1939, **74:** 297–320.
6. Green, J. D. and Harris, G. W.: A note on the blood supply and nerve supply of the hypophysis cerebri. J. Anat., 1946, **80:** 247 (Proceedings).
7. Green, J. D. and Harris, G. W.: The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocr., 1947, **5:** 136–146.
8. Green, J. D.: The adenohypophysis and the central nervous system. Bull. Blain Hosp., 1946, **5:** 186–193.
9. McConnell, E. M.: The arterial supply of the human hypophysis cerebri. Anat. Rec., 1953, **115:** 175–203.
10. Xuerb, G. P., Prichard, M. M. L. and Daniel, P. M.: The hypophyseal portal system of vessels in man. Quart. J. Exper. Physiol., 1954, **39:** 219–229.
11. Houssay, B. A., Biasotti, A. and Sammartino, R.: Modifications fonctionnelles de l'hypophyse après les lesions infundibulo-tubériennes chez le crapaud. C.R. Soc. Biol., 1935, **120:** 725–727.
12. Green, J. D.: Vessels and nerves of amphibian hypophyses. A study of the living

circulation and of the histology of the hypophyseal vessels and nerves. Anat. Rec., 1947, **99:** 21–54.

13. Green, J. D. and Harris, G. W.: Observation of the hypophysio-portal vessels of the living rat. J. Physiol., 1949, **108:** 359–361.
14. Hinsey, J. C.: The relation of the nervous system to ovulation and other phenomena of the female reproductive tract. Cold Spr. Harb. Symp. quant. Biol., 1937, **5:** 269–279.
15. Brooks, C. Mc.: A study of the mechanism whereby coitus excites the ovulation producing activity of the rabbits pituitary. Am. J. Physiol., 1938, **121:** 157–177.
16. Markee, J. E., Sawyer, C. H. and Hollinshead, W. H.: Activation of the anterior hypophysis by electrical stimulation in the rabbit. Endocrinology, 1946, **38:** 345–357.
17. Green, J. D.: The histology of the hypophyseal stalk and median eminence in man with special reference to blood vessels, nerve fibers and a special neurovascular zone in this region. Anat. Rec., 1948, **100:** 273–296.
18. Harris, G. W.: Neural control of the pituitary gland. Physiol. Rev., 1948, **28:** 139–179.
19. Green, J. D.: Innervation of the pars distalis of the adenohypophysis studied by phase microscopy. Anat. Rec., 1951, **109:** 99–108.
20. Green, J. D.: The comparative anatomy of the hypophysis, with special reference to its blood supply and innervation. Am. J. Anat., 1951, **88:** 225–312.
21. Green, J. D.: Comparative aspects of the hypophysis, especially of blood supply and innervation. *Ciba Foundation Colloquia on Endocrinology.* Vol. IV, Book I. The Blakiston Co., New York, 1952.
22. Harris, G. W.: Neural control of the pituitary gland. II. The adenohypophysis, with special reference to secretion of ACTH. Brit. Med. J., 1951, **2:** 627–633.
23. Harris, G. W.: The hypothalamus and regulation of ACTH secretion. *Adrenal Cortex. Transactions of the Third Conference.* Josiah Macy, Jr. Foundation, New York, 1952: 54–88.
24. DeGroot, J.: *The Significance of the Hypophyseal Portal Circulation.* Van Gorcum and Co., Assen, 1952.
25. Clark, E. R. and Clark, E. L.: Observations on changes in blood vascular endothelium in the living animal. Am. J. Anat., 1935, **57:** 385–438.
26. Brooks, F., Dragstedt, L., Warner, L. and Knisely, M. H.: Sludged blood following severe thermal burns. Arch. Surg., 1950, **61:** 387–418.
27. Morin, F. and Bottner, V.: Contributi alla conoscenza della irrorazione sanguina dell' ipofisi e dell' ipotalama di alcuni mammiferi. Morph. Jhb., 1941, **85:** 470–504.
28. Landsmeer, J. M. F.: Vessels of the rat's hypophysis. Acta anat., 1951, **12:** 82–109.

Fig. 1. Artist's composite drawing of the field routinely examined. The point of attachment of the stalk is to the left, the ventral surface of the pars distalis to the right. A: Long stalk arteriole. B: T-shaped arteriole at the periphery of the primary capillary system, C: Fine irregularly distributed capillaries.

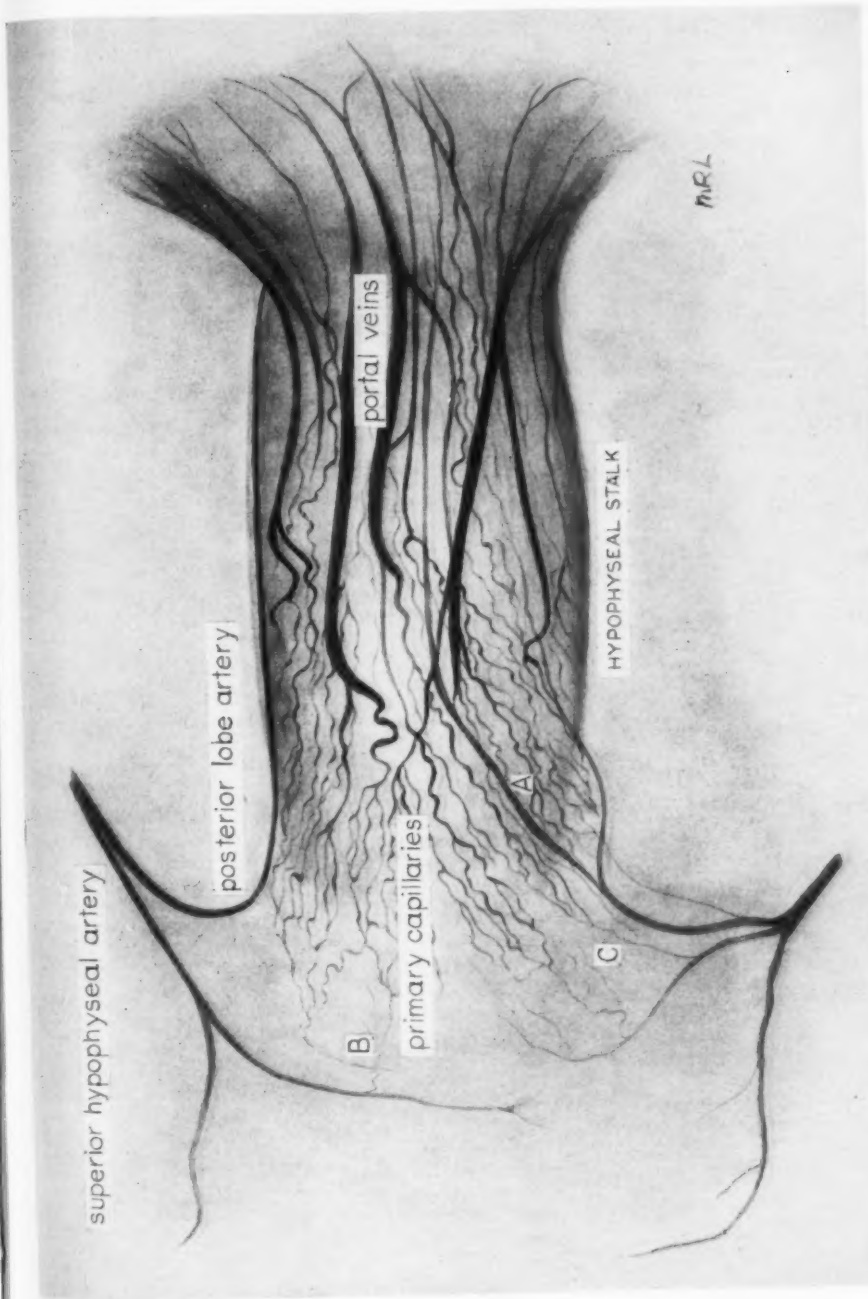

superior hypophyseal artery
posterior lobe artery
portal veins
primary capillaries
HYPOPHYSEAL STALK
A
B
C
MRL

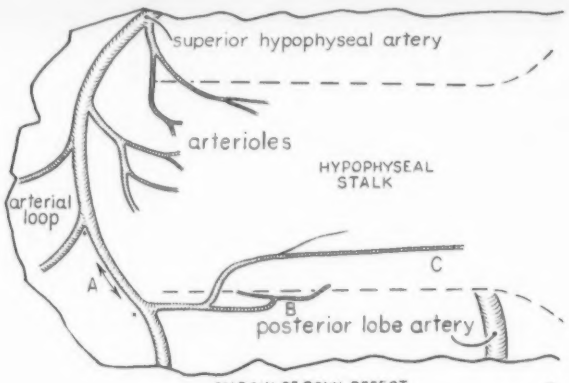
FIG. 1

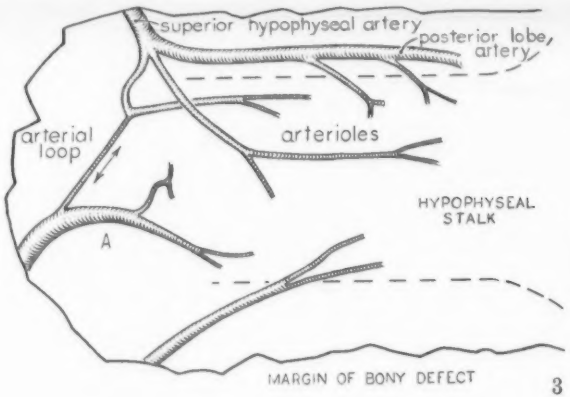
FIG. 2. A: Change in direction of flow in a segment of an arterial loop. B: T-shaped arteriole. C: Long stalk arteriole.

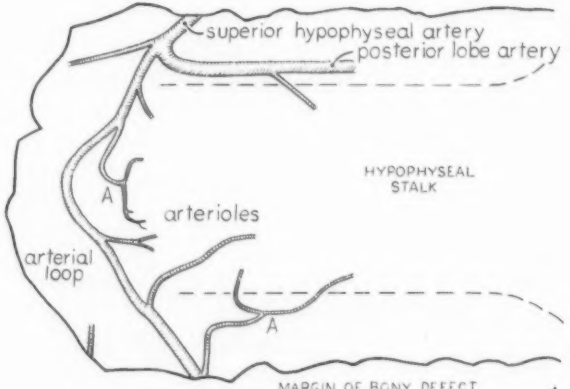
FIG. 3. A: Rapidly tapering hypophyseal artery.

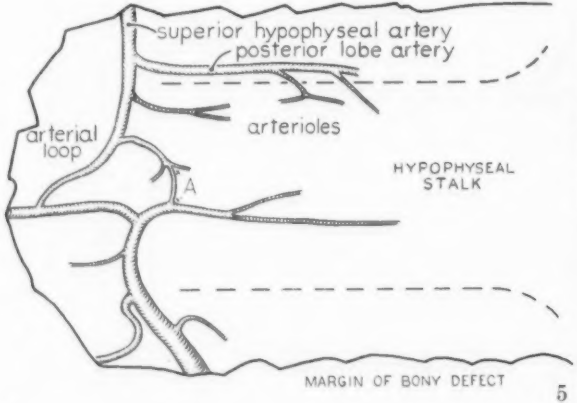
FIG. 4. A: T-shaped arteriole.

FIG. 5. Completion of an arterial loop following application of Methacholine chloride. The vessel x–x′ was not apparent before application of the drug.


superior hypophyseal artery
arterioles
HYPOPHYSEAL STALK
arterial loop
A
C
B
posterior lobe artery
MARGIN OF BONY DEFECT
2


superior hypophyseal artery
posterior lobe artery
arterioles
arterial loop
HYPOPHYSEAL STALK
A
MARGIN OF BONY DEFECT
3


superior hypophyseal artery
posterior lobe artery
HYPOPHYSEAL STALK
A
arterioles
arterial loop
A
MARGIN OF BONY DEFECT
4


superior hypophyseal artery
posterior lobe artery
arterioles
arterial loop
HYPOPHYSEAL STALK
A
MARGIN OF BONY DEFECT
5

FIGS. 2–5

FIG. 6. Group of primary capillaries. Primary capillaries and portal vein solid black. Capillary interconnections between groups, heavy double line. Intermittence of flow indicated by an asterisk. A: Fine irregularly distributed capillaries. B: Short collecting vessel.

FIG. 7. A: Wide primary capillary emerging from median eminence

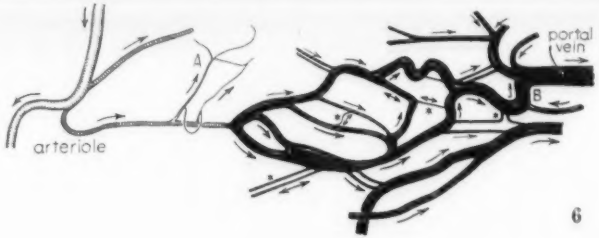
FIG. 8. A: Termination of an arteriole on a wide capillary loop.: B Fine irregularly distributed capillaries.

FIG. 9. A: Termination of an arteriole by joining a vascular space from which wide capillaries arise. B: Fine irregularly distributed capillaries.

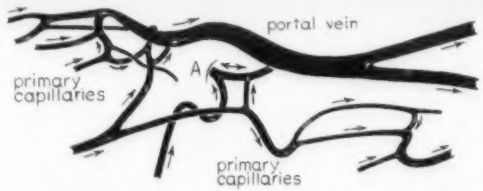
FIG. 10. A: Bifurcation of a short collecting vessel to supply two portal veins.

FIG. 11. Portal anastomosis.

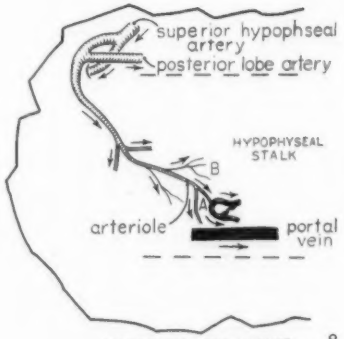
FIG. 12. A: Collecting vessel emerging from the median eminence.



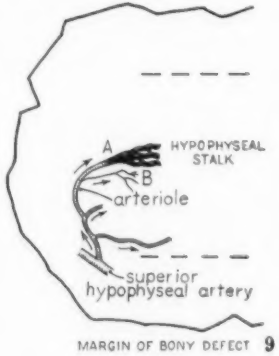
6



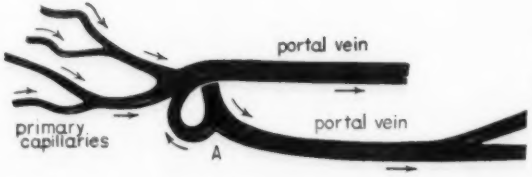
7



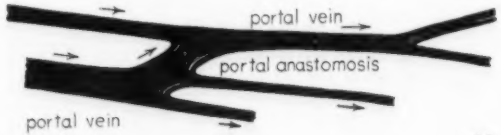
8



9



10



11

12

FIGS. 6-12

THE FUNCTION OF THE PITUITARY STALK*

G. W. HARRIS, M.D., Sc.D., F.R.S.

Fitzmary Professor of Physiology, University of London, Maudsley Hospital, England

Received for publication June 14, 1955

Mr. Chairman, Ladies and Gentlemen,

I should like to say how deeply I appreciate the honour and the responsibility of being invited to give a lecture under the auspices of The Herter Fund. One thought that sustains me at the moment is that I feel Dr. Herter, if he were with us today, would have been keenly alive to the problems now confronting so many workers in the field of neuroendocrinology. The diseases of the nervous system formed Dr. Herter's earliest scientific interest. One has only to remember the broad biological and philosophical approach that he brought to bear on questions in pathology to imagine his interest if, for example, confronted with a case of pubertas praecox associated with a hypothalamic tumour. Such cases as these present, from the clinical aspect, one of the fundamental problems of the relationship between the endocrine and central nervous systems.

Although it has been known for some years that the nervous system may profoundly influence, and be affected by, the activity of many of the endocrine glands, the mechanism by which such actions are brought about has only recently received attention. Since the pituitary gland is attached to the base of the brain by the hypophysial stalk, and since this gland in turn controls a major group of the other organs of internal secretion, interest has been aroused in the possibility that the hypophysial stalk mediates neural influences over the hypophysis and therefore indirectly over its so-called target organs.

The earliest views of the function of the pituitary stalk and gland were those expressed by the Greeks. They believed that blood, mixed with air in the left ventricle of the heart whereby it was imbued with "vital spirit", was transported to the brain where it was endowed with "animal spirit". The waste products of this latter reaction were thought to pass through the funnel-shaped infundibulum or stalk to the pituitary gland, from which the "pituita" or nasal mucus passed by ducts to the nasal cavity.

The above view was hardly replaced by any concrete advances until almost the beginning of the present century, when reports began to appear regarding the pharmacological activities of extracts of the posterior pituitary gland. In 1895 Oliver and Schäfer described the pressor activity of pituitary extract and three years later Dr. Howell, of the Johns Hopkins Hospital, located this activity in extracts of the posterior, rather than the anterior, lobe of the

*A lecture under the auspices of The Herter Lecture Fund given March 30, 1955.

gland. Further reports appeared within the next few years describing the activity of posterior lobe extracts in producing a diuresis (Magnus and Schäfer, 1901), uterine contraction (Dale, 1909), stimulation of intestinal peristalsis (Bell, 1909) and milk ejection (Ott and Scott, 1910). [It is of interest that Schäfer, who was so prominent a figure in these early studies, was Herter lecturer during the lifetime of Dr. Herter.] Following the independent work of von den Velden and Farmi in 1913, who showed that injection of posterior pituitary extract into the conscious human resulted in inhibition of a water diuresis, it seemed clear that the posterior pituitary was a gland of internal secretion, that its secretory product exerted an antidiuretic effect and that damage to the gland resulted in the clinical condition of diabetes insipidus. Since this time however two major problems have faced students of the neurohypophysis, and since two very similar problems are nowadays apparent in regard to the adenohypophysis, they will be discussed here and their probable elucidation mentioned.

THE PITUITARY STALK AND THE NEUROHYPOPHYSIS

(*a*) *The relationship of the posterior pituitary gland to the nervous system*

In 1920 Camus and Roussy and in 1921 Bailey and Bremer demonstrated that injury to the hypothalamus, in the presence of an intact pituitary gland, could give rise to experimental diabetes insipidus in the dog. Further work on other forms than dogs, such as the studies of Dr. Richter (see Richter, 1938) on rats, confirmed that a purely neural lesion might be responsible for a chronic state of polyuria. Two schools of thought then became current regarding this condition in the human, that diabetes insipidus was due to damage to the hypothalamus or that it was due to damage to the posterior pituitary gland. These apparently conflicting views were finally reconciled by the work of Ranson and his colleagues in Chicago (Fisher, Ingram and Ranson, 1938) when they found that lesions of either the anterior tuber cenereum or of the posterior pituitary gland were equally effective in producing polyuria, and put forward the view that the hypothalamus and the gland function as a unit—the two structures being linked together by the rich bundle of unmyelinated nerve fibres known as the supraopticohypophysial tract. Cajal (1894) had first described this tract in two-day-old mice, but little attention was paid to its functional significance until the work of the Ranson group became known. Evidence has now accumulated that lesions which damage a sufficient number of fibres in this tract, whether placed in the hypothalamus, or in the upper part of the neurohypophysis, inevitably result in diabetes insipidus. In this respect then the posterior pituitary gland can be compared with a voluntary muscle in that both structures are dependent on their innervation for any functional activity, even to the extent that both undergo atrophy if denervated.

Further support for the views of Ranson regarding the functional unity of the hypothalamus and neurohypophysis have been derived from studies in which the supraopticohypophysial tract has been submitted to electrical stimulation. This experiment was most easily performed on conscious animals since many of the activities of posterior pituitary extracts were known to be decreased or abolished under conditions of anaesthesia. However, using the remote control method of stimulation (Harris, 1947) it was possible to demonstrate that many of the actions attributed to the posterior pituitary gland could be elicited by stimulation of the supraopticohypophysial tract whether in the tuber cinereum, pituitary stalk or neural lobe of the gland. Inhibition of a water diuresis, increase in urinary chloride, contraction of the uterus and an increase in intestinal peristalsis were all observed to follow stimulation if the electrode was in, or within ½ mm of, the supraopticohypophysial tract. Of more recent years stimulation of the same tract has been shown (Andersson, 1951; Cross and Harris, 1952) to result in milk-ejection from the mammary gland, and again the response has been found independent of whether stimulation of the tract has been performed in the hypothalamus or in the infundibular stem.

If the supraopticohypophysial tract forms—in the Sherringtonian sense—a final common pathway to the neurohypophysis, the question may well be asked, how is excitation of the tract normally brought about, and under what physiological states are the tract and its gland activated? As Verney (1947) has shown, the supraopticohypophysial system is capable of being stimulated by changes in the osmotic pressure of the blood, a hyper-tonicity of the blood resulting in increased secretion of antidiuretic hormone. The system also seems capable of responding to nervous reflex excitation. Thus painful or other types of sensory stimuli which give rise to emotional excitement may result in secretion of the antidiuretic hormone, and excitation of sensory pathways arising from end-organs in the nipple or genital tract seem to evoke oxytocic secretion. It is probable that these latter reactions play a part in the process of milk ejection during nursing, in eliciting full uterine contractions during parturition and in increasing uterine motility and thus aiding the ascent of sperm through the female genital tract following mating. Therefore in answer to the above questions it may be suggested that both humoral and nervous reflex factors play a part in exciting the supraopticohypophysial system.

(*b*) *The relationship of neurosecretory phenomena to the neurohypophysis*

A serious problem posed by the posterior pituitary gland in the early days concerned the site of formation of the posterior pituitary hormones. Following the histological studies of Herring the view was generally current, and accepted even by such authorities as Harvey Cushing, that the posterior lobe hormones

were formed in the pars intermedia (or pars distalis) of the gland, were transported posteriorly into the neural lobe and thence up the pituitary stalk to act on nerve centres in the hypothalamus. However, data derived from studies made on extracts of the anterior and posterior lobes of various mammals (such as whales), in which the two lobes of the gland are separated by a thick connective tissue septum, showed that the pharmacological activity of so-called posterior lobe extracts was present in the neurohypophysis but not in the adenohypophysis. Search was again instituted for the secretory elements in the neural lobe itself. Various cells in the posterior lobe were from time to time suggested as secretory, for example the 'parenchymatous glandular cells' described by Gersh (1939). However no good evidence was brought forward in support of these views.

A new line of approach was opened by the observation of Bargmann (1949) that the posterior pituitary gland and its nerve supply stain almost specifically, as far as the central nervous system is concerned, by the Gomori chrome-haematoxylin phloxine method. Experimental work using this staining method later demonstrated that the intensity of the staining reaction shown by the supraopticohypophysial system varied with the hormone content of the neurohypophysis, viz., under conditions such as prolonged dehydration, in which the antidiuretic hormone content of the gland is depleted, the intensity of the staining reaction is diminished. The suggestion was then made that the site of origin of posterior pituitary hormone is in nuclear groups in the hypothalamus and that the hormone is transported down the nerve tracts in the pituitary stalk, to be liberated into the blood stream in the neurohypophysis. On this view the supraoptic and paraventricular nuclei in the hypothalamus constitute neurosecretory elements responsible for forming the hormone or its precursor, whilst the posterior pituitary gland functions as a store-house and as a site where the hormone is eventually discharged into the blood. Although further confirmation of this hypothesis is awaited, it may be said that it affords an explanation of observations difficult to harmonize on other views, such as the absence of secretory cells in the neural lobe and the fact that appreciable amounts of posterior pituitary hormone are extractable from the hypothalamus.

In summary therefore it may be suggested that the pituitary stalk forms an anatomical and functional link between the hypothalamus and posterior pituitary gland, and that through the intervention of this structure the activity of the neurohypophysis is adjusted to changes in both the external and internal environment. It may also be argued that the supraopticohypophysial tract in the stalk performs a twofold function, of transporting the posterior pituitary hormones or their precursors, from the site of formation in the hypothalamus to the site of storage and liberation in the neural lobe, and of regulating the release of the hormones into the blood streams by means of impulses passing down the nerve fibres of the tract.

THE PITUITARY STALK AND THE ADENOHYPOPHYSIS

During the last twenty-five years evidence has accumulated that the activity of the anterior pituitary gland is markedly influenced by the central nervous system. Dr. F. H. A. Marshall drew attention to and summarized the early evidence in his Croonian lecture (Marshall, 1936). Marshall discussed the role of the external environment in regulating the breeding seasons and sexual cycle of vertebrates, and pointed out that such environmental influences as food supply, temperature, humidity, light, presence of a mate or member of the same sex may profoundly modify the sexual cycles. It is highly probable that many of these effects are brought about by central nervous reflex paths. Clear cut examples are seen in the influence which the act of mating has on the ovary in most birds and many mammals (rabbit, ferret, cat, rat, mouse, mink, ground-squirrel), and also in the part played by changes in environmental lighting conditions at the time of onset of the breeding season. In the ferret, for example, the female is normally anoestrous during the winter months, but may be brought into full reproductive activity at this time of the year by artificially lengthening the hours of illumination. This reaction is mediated by the eyes, optic nerves and some nervous reflex pathway with resultant increase in gonadotrophic secretion.

More recent work indicates that the release of the adrenocorticotrophic (ACTH) and thyrotrophic (TSH) hormones are also affected by neural factors. That emotional stress may induce increased discharge of ACTH is too well known to receive further elaboration, and an inhibition of TSH secretion contingent on similar stimuli has lately been reported. Such data afford firm ground for the belief that the central nervous system may modify the rate of secretion of these hormones.

Although secretion of the follicle stimulating, luteinizing, luteotrophic or lactogenic, adrenocorticotrophic and thyrotrophic hormones may be influenced by the nervous system, there is little information available as to the details of the mechanism involved. Early suggestions that the cervical sympathetic system, or cranial parasympathetic system (petrosal branches of the facial nerve) supplied the anterior pituitary gland with secretomotor fibres are unlikely since removal of these nerves, in certain forms such as the rat and rabbit, does not abolish reflex activation of this gland. The further suggestion that the pituitary stalk forms the intervening link between the central nervous system and the adenohypophysis by means of a direct nerve supply to the gland is also unlikely, since simple section of the pituitary stalk does not necessarily disturb anterior pituitary function. Any supposition based on a direct innervation of the anterior lobe is, in fact, negated by the histological evidence. The most detailed studies of pituitary innervation have either failed to reveal any nerve fibres in the gland (Green, 1951; Wingstrand, 1951) or a great paucity of fibres (Rasmussen, 1938).

HYPOPHYSIAL PORTAL VESSELS

In 1930 and 1933 Popa and Fielding published the first descriptions of an anatomical link connecting the hypothalamus and the adenohypophysis—the hypophysial portal system of vessels. These vessels consist of capillaries in the median eminence of the tuber cinereum which unite into vascular trunks in the region of the pituitary stalk and break up again into capillaries, or sinusoids, in the anterior pituitary. The anatomical description of these vessels was quickly confirmed by Wislocki and King (1936), and later extended by studies in other forms by Green and Harris (1947), Green (1951) and Wingstrand (1951). A recent study of these vessels in the human has been made by Xuereb, Prichard and Daniel (1954) from whose publication Figure 1 is taken. The detailed account

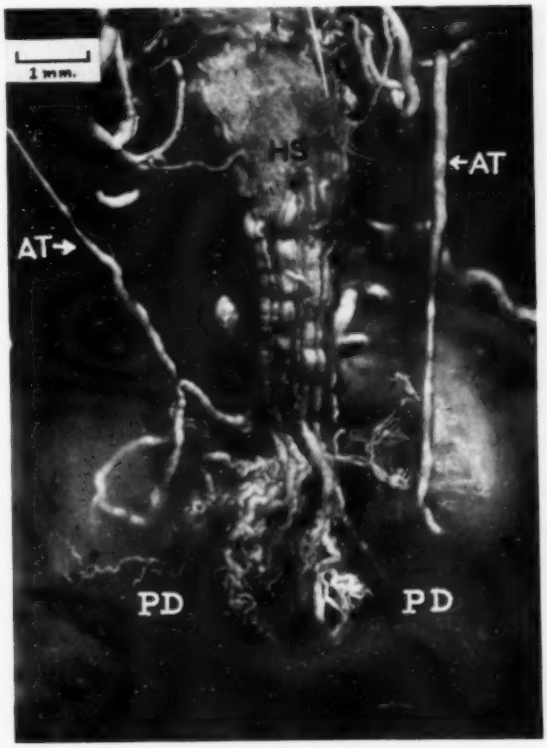


FIG. 1. Photograph of the anterior aspect of the pituitary stalk of man, in which the blood vessels have been injected with neoprene latex. Note the prominent trunks of the portal vessels with the hypophysial stalk (HS) running down into the pars distalis (PD) which has been partly dissected to show the vessels breaking up into the sinusoids of the gland. AT, systemic arteries entering the pars distalis. (From Xuereb, G. P., Prichard, Marjorie M. L. and Daniel, P. M.: Quart. J. Exp. Physiol., 1954, **39**: 219.) Published by kind permission of the authors and the Editor of the Quart. J. Exp. Physiol.

of these latter authors would seem particularly opportune at the moment, in view of the operation of hypophysectomy that is being performed in man in many clinics.

When it became realised that these vessels constitute a constant anatomical feature in many different forms the possibility was raised that in some way they link the functional activity of the hypothalamus and the anterior pituitary gland. One difficulty of such a view initially, was that the direction of blood flow in the vessels was uncertain. Popa and Fielding had surmised that the flow was from the pituitary gland to the hypothalamus. However the error of this view was established by direct microscopic observation of the blood flowing in the vessels of amphibians (Green, 1947), rats (Green and Harris, 1949) and mice (Worthington, personal communication). In this way the flow has been seen to be even and non-pulsatile, and in a direction from the tuber cinereum to the anterior pituitary gland.

The view that the pituitary stalk by means of its vascular component unites the hypothalamus and adenohypophysis into a functioning unit, in a manner similar to that in which the supraopticohypophysial tract of the stalk unites the hypothalamus and neurohypophysis, is supported by the following data.

(*a*) *The effect of electrical stimulation of the hypothalamus and anterior pituitary gland*

Electrical stimulation of the hypothalamus may result in discharge of luteinising hormone in the rabbit, as evidenced by the occurrence of ovulation. However, similar stimulation applied to the pituitary stalk or directly to the anterior pituitary gland even for much longer periods (seven and a half hours as compared with a few minutes) does not produce this result (Markee, Sawyer and Hollinshead, 1946; Harris, 1948). Similarly, stimulation of the posterior tuber cinereum or mammillary bodies may result in discharge of the adrenocorticotrophic hormone, whereas direct stimulation of the pituitary stalk or gland is ineffective (de Groot and Harris, 1950). These findings are compatible with the view that stimulation of the hypothalamus excites some part of a nervous reflex path, whereas stimulation of the pituitary stalk or gland is ineffective since the physiological stimulus is transmitted humorally in these structures.

(*b*) *The effect of pituitary stalk section on anterior pituitary function*

Few procedures have given such discordant results as those involving pituitary stalk section in studies on anterior pituitary function. Following stalk section the posterior pituitary undergoes atrophy and since its nerve supply does not regenerate any functional activity of the gland ceases indefinitely. In view of this clear cut result it was puzzling that some observers reported normal anterior lobe activity after similar surgical interference, although others re-

ported marked signs of anterior pituitary deficiency. A possible explanation of these variable results was that in the hands of some operators section of the pituitary stalk might be followed by vascular regeneration across the site of section and therefore reestablishment of normal anterior pituitary function, whereas in other hands various details of surgical technique might act to prevent such regeneration and so result in anterior pituitary hypofunction. To test this hypothesis the pituitary stalk was cut in a series of rats and in some animals a plate of paper was placed between the cut ends of the stalk (Harris, 1950). The results of this work revealed, firstly that the hypophysial portal vessels of the rat possess marked and rapid regenerative capacities; secondly, that a large paper plate placed between the ends of the stalk forms an effective obstacle to such regeneration; and thirdly, that there is a correlation between portal vessel regeneration and the return of oestrous cycles and the pseudopregnancy response to sterile mating. Regeneration of the portal vessels has now been seen to occur also in the mouse, rabbit, ferret and monkey. A recent study (Donovan and Harris, 1954) in the ferret has shown a correspondence between vascular regeneration across the site of stalk section and the return of gonadotrophic secretion similar to that in the rat. The criterion of gonadotrophic secretion taken in this work was the onset of oestrus following extra illumination during winter. It was found that in five out of six animals in which the stalk had been simply divided the portal vessels regenerated and the animals showed an oestrous response to the extra illumination. Out of fourteen ferrets in which the stalk was cut and a paper plate inserted between the cut ends only three showed a response—and in these three animals microscopic examination revealed that the plates had been misplaced and that vascular regeneration had occurred around the borders of the plate. The conclusion drawn was that the vessels of the pituitary stalk form part of a neurohumoral reflex path between the sensory receptors in the retina and the anterior pituitary gland. It should be mentioned however that Thomson and Zuckerman (1954) claim that ferrets in which the pituitary stalk has been cut, and in which the portal vessels have not regenerated, may still show the light-induced oestrous response. They base their view on results obtained in three animals. It is felt that the histological methods used by these workers may have been inadequate to reveal the vascular pattern of the area involved (see Donovan and Harris, 1954) and that the oestrous ferrets studied in this work did in fact possess vascular connections between the tuber cinereum and adenohypophysis. Recently Abrams, Marshall and Thomson (1954) have reported that cervical sympathectomy is effective in preventing the oestrous response to light in the ferret. It is difficult to argue from these findings that the cervical sympathetic chain forms part of a nervous reflex arc regulating anterior pituitary activity, since there is much evidence in other forms that cervical or complete sympathectomy has little effect on pituitary function. Further Donovan and v.d. Werff t. Bosch (personal communication),

who repeated the above study, found that bilateral removal of the superior cervical ganglia or the stellate ganglia did not abolish the development of oestrus following extra lighting in winter, but that this state took longer to develop than in the control animals. A likely explanation of these results is that cervical sympathectomy, which in the ferret as in other forms results in constriction of the pupil, ptosis and enophthalmos, affects the oestrous response by decreasing the sensitivity of the sensory end organ (i.e., the eye) rather than by breaking a reflex arc.

(*c*) *The effect of pituitary transplantation on anterior pituitary function*

Although the ovary, testis, adrenal cortex and thyroid may be transplanted to a distant site in the body and maintain good functional activity, many studies of transplantation of anterior pituitary tissue have failed to reveal similar results. A possible explanation of these findings was that the glands in the former group are stimulated by trophic hormones which would reach them, even in distant regions, via the systemic circulation, whereas the anterior pituitary gland might be deprived of its normal stimulus to activity if placed in a site remote from the hypophysial portal circulation. Evidence confirming this view was forthcoming when it was found (Harris and Jacobsohn, 1952) that anterior pituitary tissue placed beneath the tuber cinereum of hypophysectomized female rats became revascularized by the portal vessels in a high proportion of cases and showed apparently normal functional activity. In control experiments transplants placed beneath the temporal lobe of the brain were found to become well vascularized (though not by the portal system) but failed to show any signs of activity. The conclusion drawn from these observations was that a portal blood supply was the essential factor underlying normal activity of anterior pituitary tissue so far at least as the secretion of gonadotrophic, thyrotrophic and adrenocorticotrophic hormones are concerned. It was also observed during the course of the above study that anterior pituitary tissue obtained from new born rats and transplanted beneath the tuber cinereum of adult female hosts was capable of maintaining adult pituitary function within a few days of transplantation, and that anterior pituitary tissue from male rats placed into adult females could maintain the female reproductive functions of oestrous cycles, pregnancy and milk secretion. These results show that the development of puberty is not dependent on an "ageing" of the pituitary, and that the type of activity shown by the female pituitary is not dependent on any inherent property of the gland. The facts indicate, rather, the plastic nature of anterior pituitary tissue and emphasize the dependence of its pattern of activity on its anatomical connections with the hypothalamus. Further, they throw open for future investigation the part played by the central nervous system in the onset of puberty, a question raised by certain cases of pubertas praecox seen in the human.

It is also interesting to speculate on a possible correlation between the following:—

(a), Secondary growths arising from primary malignant tumours in the ovary, testis, thyroid and adrenal cortex are common, but in the case of primary growths of the pituitary metastatic deposits are very rare (see King, 1951).

and

(b), It is relatively easy to obtain a good degree of functional activity in transplants of the ovary, testis, thyroid and adrenal cortex, but difficult or impossible to obtain similar activity in pituitary transplants placed in sites remote from the sella turcica.

If a correlation does exist between the above observations, it might imply that the initiation of growth of metastatic tumours requires the presence of some factor normally concerned with the activation of the tissue or organ in which the primary growth is situated.

DEGREE OF DEPENDENCE OF ADENOHYPOPHYSIAL FUNCTION ON THE HYPOTHALAMUS

There would seem to be little doubt that the hypophysial portal vessels are intimately related in some way to anterior pituitary function. This view however raises further problems, such as—is the hypothalamus *entirely* responsible for maintaining the activity of the adenohypophysis (as is the case for the neurohypophysis)? And, do environmental stimuli of a stressful or noxious type act on the anterior pituitary in other ways than through the pituitary stalk? In order to answer these questions a study has been made of the activity of the thyroid and adrenal glands in rabbits in which the pituitary stalk has been sectioned, and a large plate of waxed paper placed to intervene between the hypothalamus and gland. In this work it was found (Fortier, Harris and McDonald, unpublished) that the adrenal glands undergo marked atrophy though they remain slightly larger than the glands of hypophysectomized animals. The "denervated" pituitary was found to release ACTH in response to stress stimuli of a type which may be presumed to act via the general systemic circulation ("systemic stresses"—injection of adrenaline, trauma of laparotomy) but the response to stresses which possibly act solely via the central nervous system ("neural stresses"—emotional stress of restraint, exposure to cold) was abolished. Similar results were observed (Brown-Grant, Harris and Reichlin, unpublished) on the same animals when the activity of the thyroid was studied using radioactive iodine. Pituitary stalk section resulted in a reduction of thyroid activity though not to such a low level as follows hypophysectomy, did not abolish the thyroid inhibition that normally follows administration of thyroxine or laparotomy, but did abolish the inhibition that normally follows restraint or injection of stilboestrol. The claim that the release of

ACTH in response to surgical trauma is abolished by hypothalamic lesions (Hume, 1953; McCann, 1953; Porter, 1953) is difficult to reconcile with the present results. A possible explanation may be found in a decreased sensitivity of the adrenal cortex to ACTH following lesions of the median eminence, and some recent findings (Fortier, Harris and McDonald, unpublished) would support this view. The results obtained on the stalk-sectioned rabbit however are in general agreement with the earlier results of Fortier (1951) and support the concept that the anterior pituitary gland removed from the influence of the central nervous system does maintain a low level of activity, and that this activity can be affected by procedures that result in some change in the composition of the general systemic blood. From this standpoint the influence of the hypothalamus and pituitary stalk on anterior pituitary function is to maintain such function at a normal high level, and to regulate this activity according to the requirements imposed by a constantly changing external environment.

MECHANISM OF ACTION OF THE HYPOPHYSIAL PORTAL VESSELS

Although a blood supply derived from the hypophysial portal vessels is clearly related to the maintenance and regulation of anterior pituitary activity, the mechanism by which such effects are exerted is unknown. The present data are most easily explained by the postulation that a humoral substance (or substances) is liberated by hypothalamic nerve fibres into the primary plexus of the vessels in the median eminence, carried down the stalk to the anterior lobe and there excites or inhibits the activity of the glandular cells. Work is in progress in many laboratories at the present time, using both in vitro and in vivo methods, investigating this possibility. Several suggestions have been put forward as to the nature of a transmitter substance, but such suggestions and the neurohumoral view as a whole will only be established if it is possible to firstly identify a particular substance which exerts a direct action on anterior pituitary cells; secondly, to show this substance is present in the blood in the hypophysial portal vessels in greater amount than in systemic blood; thirdly, to show that the concentration of this substance in the blood of the hypophysial portal vessels varies according to electrical or reflex activation of hypothalamic nerve tracts; and fourthly, to demonstrate that the activity of the adenohypophysis is correlated with this varying concentration. Such data as this are not yet available for any of the substances that might play the role of transmitter agent (adrenergic substance—Markee, Sawyer and Hollinshead, 1948; Sawyer, Markee and Everett, 1950; neurosecretory material associated with the neurohypophysis—Benoit and Assenmacher, 1953; Palay, 1953; Rothballer, 1953; posterior pituitary hormone—Mirsky, Stein and Paulisch, 1954; lipide or lipoprotein present in hypothalamic extracts—Slusher and Roberts, 1954; histamine—Harris, Jacobsohn and Kahlson, 1952; and other substances.

In view of the possibility that the portal vessels of the stalk function as part

of a neurohumoral path regulating the adenohypophysis, two points of comparison may be drawn between this gland and the neurohypophysis. Firstly—the argument as to whether the essential lesion underlying a state of diabetes insipidus is one involving the hypothalamus or posterior pituitary gland was finally resolved when it became apparent that these structures function as a unit knit together by the nerve fibres of the pituitary stalk. In a similar manner, the fact that hypothalamic lesions may disturb anterior pituitary function is explainable on the grounds that this region of the brain and the adenohypophysis are functionally associated by the portal vessels of the stalk. And secondly—close comparison may be made between the phenomenon of neurosecretion as put forward to describe the formation of posterior pituitary hormones and the suggested neurohumoral regulation of the anterior pituitary. Both these views envisage the transfer of chemical substances from nerve terminals into the blood stream. In both cases the transfer would occur within the confines of the neurohypophysis: posterior pituitary hormones enter the blood stream in all three parts of the neurohypophysis (the median eminence of the tuber cinereum, the infundibular stem and the infundibular process), and the transfer to the primary plexus of portal vessels would occur in the median eminence and, in some forms, in the infundibular stem. In this respect it is perhaps noteworthy that the permeability of the vessels of the neurohypophysis appears to be different from that of the vessels of the hypothalamus and other parts of the central nervous system (Wislocki and King, 1936).

LOCALIZATION OF PITUITARY FUNCTION IN THE HYPOTHALAMUS

It is possible that the control of the secretion of the different pituitary hormones is represented by different mechanisms and areas in the hypothalamus in a manner similar to that in which the parts of the body are represented in the motor and sensory cortex of the cerebrum. It would seem unlikely, if this conjecture is in any way true, that future work will result in a single picture since a multitude of neural paths from remote parts of the nervous system pass through the hypothalamus and many of these may produce varied effects on pituitary activity. However from the meagre information available at the moment lesions in, or electrical stimulation of, certain regions of the hypothalamus have been found to result in definite changes in pituitary secretion. It appears certain in some cases, and probable in others, that these changes are mediated by the "final common pathway" of the pituitary stalk. The present position may be briefly summarized as in Figure 2 and as follows:—

(a) Posterior pituitary hormones. There is overwhelming evidence that the supraoptic nucleus and supraopticohypophysial tract are concerned with the secretion of antidiuretic hormone. Preliminary accounts (Cross, personal communication; Olivecrona, 1954) suggest that the paraventricular nucleus may be associated with the secretion of oxytocic hormone.

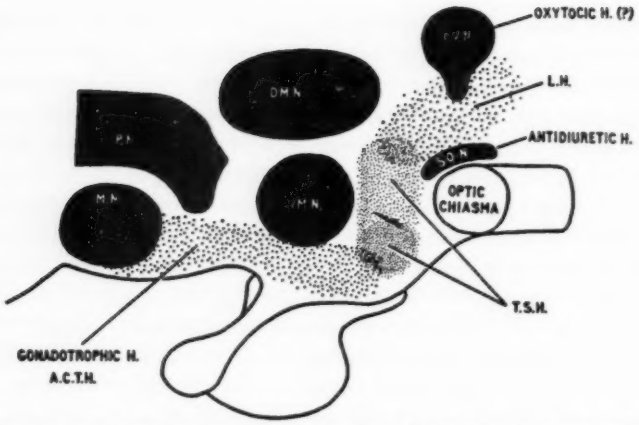


FIG. 2. Diagram of a midline sagittal section through the hypothalamus and pituitary gland. Various hypothalamic nuclei (DM.N., dorso-medial nucleus; M.N., mammillary nuclei; P.N., posterior nucleus; PV.N., paraventricular nucleus; SO.N., supraoptic nucleus; VM.N., ventromedial nucleus) are indicated in black. The stippled areas indicate the sites where electrical stimulation or lesions have resulted in changes of pituitary secretion.

(b) Gonadotrophic hormones. Stimulation of the tuber cinereum, especially the more posterior part, in rabbits has been found to result in release of luteinising hormone and ovulation (Markee, Sawyer and Hollinshead, 1946; Harris, 1948).

Lesions of the median eminence of the tuber cinereum in guinea-pigs and rats result in gonadal atrophy (Dey, Fisher, Berry and Ranson, 1940; Dey, 1943; McCann, 1953). Lesions in the hypothalamus more remote from the region of the pituitary stalk—especially those lying anterior and ventral to the paraventricular nucleus (Dey, Fisher, Berry and Ranson, 1940; Dey, 1943; Hillarp, 1949; Greer, 1953) or involving the ventromedian nucleus (Barrnett and Mayer, 1954)—disturb the release of luteinising hormone in rats and result in a condition of constant oestrus. Small localized lesions, generally in the posterior tuber cinereum or mammillary region of the hypothalamus, in young children may result in precocious puberty, presumably through anterior pituitary activation (Bauer, 1954).

(c) Adrenocorticotrophic hormone. Electrical stimulation of the posterior tuber cinereum or mammillary bodies has been reported to cause release of ACTH in rabbits, cats and monkeys (de Groot and Harris, 1950; Porter, 1953, 1954). Stimulation of the tuberal region in dogs evokes a similar response (Hume, 1953).

Lesions in the floor of the third ventricle, especially in the tuberal or more posterior regions, have been reported by many workers to block the increased

secretion of ACTH that normally follows various types of noxious or stressful stimuli (de Groot and Harris, 1950—rabbit; Hume, 1952, 1953—dog; Porter, 1953, 1954—cat and monkey; McCann, 1953—rat; Laqueur, McCann, Schreiner, Rosemberg, Anderson and Rioch, 1953—cat). Ganong and Hume (1954) have recently reported that lesions in the median eminence of dogs abolish the compensatory adrenal hypertrophy that normally follows unilateral adrenalectomy.

(d) Thyrotrophic hormone. Lesions in the hypothalamus situated between the ventro-median and suprachiasmatic nuclei prevent the hypertrophy of the thyroid that follows thiouracil administration (Greer, 1952; Bogdanove and Halmi, 1953). Ganong, Frederickson and Hume (1954) found that lesions placed in, or just above, the anterior end of the median eminence of dogs depress the thyroid uptake of radio-iodine to within the range seen in hypophysectomized animals.

It is of interest that Bogdanove and Halmi (1953) and Ganong, Fredrickson and Hume (1954) report that hypothalamic lesions may, according to their site, affect thyrotrophic and gonadotrophic secretion independently. This implies that separate mechanisms control the secretion of these hormones at the hypothalamic level.

(e) Growth hormone. Little evidence is available that the secretion of growth hormone (STH) is influenced by the hypothalamus. The data of Spirtos, Ingram, Bogdanova and Halmi (1954) might be taken in favour of the view that some region near the paraventricular nucleus is associated with this function.

GENERAL CONCLUSIONS

It has been suggested (Harris and Fortier, 1954) that the function of the pituitary stalk may be expressed in general terms as forming a connecting link between the external environment and the central nervous system on the one hand, and the pituitary gland and its target organs on the other. Experiments in which the stalk is cut, or in which lesions are placed in the tuber cinereum, show that the activity of the neurohypophysis is entirely dependent on its connection with the hypothalamus, whereas that of the adenohypophysis is largely, but not completely, dependent on its connection with the nervous system. It is probable that the hypothalamus and pituitary stalk are responsible for regulating the changes in activity of the two lobes of the gland in accordance with the needs of the organism in an ever changing environment.

The fact that the anterior pituitary shows the ability to function at a low level of activity after pituitary stalk section, may be compared with the function of the adrenal cortex and thyroid gland after hypophysectomy. The adrenal cortex of the hypophysectomized animal still preserves sufficient output

of adrenal steroids to maintain the life of the hypophysectomized animal as compared with that of the adrenalectomized animal. Similarly, the thyroid gland still accumulates iodine and secretes thyroid hormone to some extent, after hypophysectomy. It would seem that there is inherent in the glandular tissue a low level of autonomous function. In the case of the isolated and perfused adrenal gland Vogt (1951) has shown that the cortical activity may be altered by varying the potassium/sodium ratio of the plasma, and this effect may be similar to that seen in the anterior pituitary following stalk section, when injection of adrenaline or operative trauma still results in increased discharge of ACTH.

If the pituitary stalk functions as a connecting link between the external environment and major part of the endocrine system, it is pertinent to ask whether the mechanism is functional in the foetus when the external environment is relatively constant. This question has been recently discussed (Harris, 1955), but it may be mentioned here that there is some data indicating that the hypothalamo-hypophysial system only undergoes full maturation after birth. Although the anterior pituitary, thyroid, adrenal cortex and gonads of the foetus are functional to some degree (see Jost, 1953), and their activity may be affected by changes in composition of the circulating blood (compensatory adrenal hypertrophy follows unilateral adrenalectomy in the foetus: Kitchell and Wells, 1952) it would seem that environmental changes are unable to modify the function of these glands till some time after birth (Jailer, 1950; Thompson and Blount, 1954). The full activity of the gonads, and their responsiveness to environmental stimuli, is clearly not established till puberty—and as has been pointed out above, there is evidence that the onset of this state is in some way related to the hypothalamus. There seems to be some similarity between the endocrine system of the foetus or new born animal and that of the adult following pituitary stalk section.

REFERENCES

Abrams, M. E., Marshall, W. A. and Thomson, A. P. D.: Effect of cervical sympathectomy on the onset of oestrus in ferrets. Nature, 1954, **174:** 311.

Andersson, B.: The effect and localisation of electrical stimulation of certain parts of the brain stem in sheep and goats. Acta Physiol. Scand., 1951, **23:** 8–23.

Bailey, P. and Bremer, F.: Experimental diabetes insipidus. Arch. Int. Med., 1921, **28:** 773–803.

Bargmann, W.: Uber die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophyse. Z. Zellforsch., 1949, **34:** 610–634.

Barrnett, R. J. and Mayer, J.: Endocrine effects of hypothalamic lesions. Anat. Rec., 1954, **118:** 374–375.

Bauer, H. G.: Endocrine and other clinical manifestations of hypothalamic disease. J. Clin. Endocrinol., 1954, **14:** 13–31.

Bell, W. B.: The pituitary body and the therapeutic value of the infundibular extract in shock, uterine atony and intestinal paresis. Brit. Med. J., 1909, **2:** 1609–1613.

BENOIT, J. AND ASSENMACHER, I.: Rapport entre la stimulation sexuelle préhypophysaire et la neurosécrétion chez l'oiseau. Arch. anat. micr., 1953, **42:** 334–386.

BOGDANOVE, E. M. AND HALMI, N. S.: Effect of hypothalamic lesions and subsequent propylthiouracil treatment on pituitary structure and function in the rat. Endocrinology, 1953, **53:** 274–292.

CAJAL, S. R.: Algunas contribuciones al conocimiento del cerebro. III. Hypophysis. An. Soc. Esp. Hist. Nat. 2 a series, t.3, 1 de Agosto de 1894 (cited from *Histologie du système nerveux de l'Homme et des Vertébrés*. S. R. Cajal, French Translation, Paris, 1911. A. Maloine, éditeur.)

CAMUS, J. AND ROUSSY, G.: Experimental researches on the pituitary body. Diabetes insipidus, glycosuria and those dystrophies considered as hypophyseal in origin. Endocrinology, 1920, **4:** 507–522.

CROSS, B. A. AND HARRIS, G. W.: The role of the neurohypophysis in the milk-ejection reflex. J. Endocrinol., 1952, **8:** 148–161.

DALE, H. H.: The action of extracts of the pituitary body. Biochem. J., 1909, **4:** 427–447.

DEY, F. L.: Evidence of hypothalamic control of hypophyseal gonadotropic function in the female guinea-pig. Endocrinology, 1943, **33:** 75–82.

DEY, F. L., FISHER, C., BERRY, C. M. AND RANSON, S. W.: Disturbances in reproductive functions caused by hypothalamic lesions in female guinea-pigs. Am. J. Physiol., 1940, **129:** 39–46.

DONOVAN, B. T. AND HARRIS, G. W.: Effect of pituitary stalk section on light-induced oestrus in the ferret. Nature, 1954, **174:** 503–504.

FARMI, F. VON.: Ueber Diabetes insipidus und Hypophysistherapie. Gaz. degli. Osped. Nr. 109 (Cited from Wien klin. Woch., 1913, **26:** 1867.)

FISHER, C., INGRAM, W. R. AND RANSON, S. W.: *Diabetes Insipidus* etc., Edwards Bros., Ann Arbor, Michigan. 1938.

FORTIER, C.: Dual control of adrenocorticotrophin release. Endocrinology, 1951, **49:** 782–788.

GANONG, W. F., FREDRICKSON, D. S. AND HUME, D. M.: Depression of the thyroidal iodine uptake by hypothalamic lesions. J. Clin. Endocrinol., 1954, **14:** 773–774.

GANONG, W. F. AND HUME, D. M.: Absence of stress-induced and "compensatory" adrenal hypertrophy in dogs with hypothalamic lesions. Endocrinology, 1954, **55:** 474–483.

GERSH, I.: The structure and function of the parenchymatous glandular cells in the neurohypophysis of the rat. Am. J. Anat., 1939, **64:** 407–443.

GREEN, J. D.: Vessels and nerves of amphibian hypophyses: A study of the living circulation and of the histology of the hypophysial vessels and nerves. Anat. Rec., 1947, **99:** 21–54.

GREEN, J. D.: The comparative anatomy of the hypophysis, with special reference to its blood supply and innervation. Am. J. Anat., 1951, **88:** 225–312.

GREEN, J. D. AND HARRIS, G. W.: The neurovascular link between the neurohypophysis and adenohypophysis. J. Endocrinol., 1947, **5:** 136–146.

GREEN, J. D. AND HARRIS, G. W.: Observation of the hypophysio-portal vessels of the living rat. J. Physiol., 1949, **108:** 359–361.

GREER, M. A.: The role of the hypothalamus in the control of thyroid function. J. Clin. Endocrinol., 1952, **12:** 1259–1268.

GREER, M. A.: The effect of progesterone on persistent vaginal estrus produced by hypothalamic lesions in the rat. Endocrinology, 1953, **53:** 380–390.

DE GROOT, J. AND HARRIS, G. W.: Hypothalamic control of the anterior pituitary gland and blood lymphocytes. J. Physiol., 1950, **111:** 335–346.

HARRIS, G. W.: The innervation and actions of the neurohypophysis; an investigation using the method of remote control stimulation. Philos. Trans. B., 1947, **232:** 385–441.

HARRIS, G. W.: Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. J. Physiol., 1948, **107:** 418–429.

HARRIS, G. W.: Oestrous rhythm, pseudopregnancy and the pituitary stalk in the rat. J. Physiol., 1950, **111:** 347–360.

HARRIS, G. W.: The relationship between endocrine activity and the development of the nervous system. The First International Neurochemical Congress, Oxford. Academic Press Inc., New York, 1955, in press.

HARRIS, G. W. AND FORTIER, C.: The regulation of anterior pituitary function, with special reference to the secretion of adrenocorticotrophic hormone. Pages 106–127 in the *Fourth Annual Report on Stress*, Selye, H., and Heuser, G. Acta Inc., Medical Publishers, Montreal, 1954.

HARRIS, G. W. AND JACOBSOHN, DORA: Functional grafts of the anterior pituitary gland. Proc. Roy. Soc. London B., 1952, **139:** 263–276.

HARRIS, G. W., JACOBSOHN, DORA AND KAHLSON, G.: The occurrence of histamine in cerebral regions related to the hypophysis. Ciba Foundation Colloquia on Endocrinology, 1952, **4:** 186–193.

HILLARP. N.-A.: Studies on the localization of hypothalamic centres controlling the gonadotrophic function of the hypophysis. Acta endocrinol., 1949, **2:** 11–23.

HOWELL, W. H.: The physiological effects of extracts of the hypophysis cerebri and infundibular body. J. Exp. Med., 1898, **3:** 245–258.

HUME, D. M.: The relationship of the hypothalamus to the pituitary secretion of ACTH. Ciba Foundation Colloquia on Endocrinology, 1952, **4:** 87–99.

HUME, D. M.: The neuroendocrine response to injury: present status of the problem. Ann. Surg., 1953, **138:** 548–557.

JAILER, J. W.: The maturation of the pituitary-adrenal axis in the newborn rat. Endocrinology, 1950, **46:** 420–425.

JOST, A.: Problems of fetal endocrinology: the gonadal and hypophyseal hormones. Recent Prog. in Horm. Res., 1953, **8:** 379–413.

KING, A. B.: The diagnosis of carcinoma of the pituitary gland. Bull. Johns Hopkins Hosp., 1951, **89:** 339–353.

KITCHELL, R. L. AND WELLS, L. J.: Functioning of the hypophysis and adrenals in fetal rats. Anat. Rec., 1952, **112:** 561–591.

LAQUEUR, G. L., MCCANN, S. M., SCHREINER, L. H., ROSEMBERG, E., ANDERSON, E. AND RIOCH, D. M.: Morphology and function of the adrenal cortex in cats with hypothalamic lesions. Abstr. XIX Internat. Physiol. Congress, Montreal, 1953, p. 542.

MAGNUS, R. AND SCHÄFER, E. A.: The action of pituitary extracts upon the kidney. J. Physiol., 1901, **27:** ix–x.

MARKEE, J. E., SAWYER, C. H. AND HOLLINSHEAD, W. H.: Activation of the anterior hypophysis by electrical stimulation in the rabbit. Endocrinology, 1946, **38:** 345–357.

MARKEE, J. E., SAWYER, C. H. AND HOLLINSHEAD, W. H.: Adrenergic control of the release of luteinizing hormone from the hypophysis of the rabbit. Recent Prog. in Horm. Res., 1948, **2:** 117–131.

MARSHALL, F. H. A.: Sexual periodicity and the causes which determine it. Philos. Trans. B., 1936, **226:** 423–456.

MCCANN, S. M.: Effect of hypothalamic lesions on the adrenal cortical response to stress in the rat. Am. J. Physiol., 1953, **175:** 13–20.

MIRSKY, I. A., STEIN, M. AND PAULISCH, G.: The secretion of an antidiuretic substance into the circulation of adrenalectomized and hypophysectomized rats exposed to noxious stimuli. Endocrinology, 1954, **55:** 28–39.

OLIVECRONA, H.: Relation of the paraventricular nucleus to the pituitary gland. Nature, 1954, **173:** 1001.

OLIVER, G. AND SCHÄFER, E. A.: On the physiological action of extracts of pituitary body and certain other glandular organs. J. Physiol., 1895, **18:** 277–279.

OTT, I. AND SCOTT, J. C.: The action of infundibulin upon the mammary secretion. Proc. Soc. Exp. Biol. and Med., N. Y., 1910, **8:** 48–49.

PALAY, S. L.: Neurosecretory phenomena in the hypothalamohypophysial system of man and monkey. Amer. J. Anat., 1953, **93:** 107–127.

POPA, G. T. AND FIELDING, U.: A portal circulation from the pituitary to the hypothalamic region. J. Anat., Lond., 1930, **65:** 88–91.

POPA, G. T. AND FIELDING, U.: Hypophysio-portal vessels and their colloid accompaniment. J. Anat., Lond., 1933, **67:** 227–232.

PORTER, R. W.: Hypothalamic involvement in the pituitary-adrenocortical response to stress stimuli. Am. J. Physiol., 1953, **172:** 515–519.

PORTER, R. W.: The central nervous system and stress-induced eosinopenia. Rec. Prog. in Horm. Res., 1954, **10:** 1–18.

RASMUSSEN, A. T.: Innervation of the hypophysis. Endocrinology, 1938, **23:** 263–278.

RICHTER, C. P.: The pituitary gland in relation to water exchange. Proc. Assn. Res. Nerv. and Ment. Dis., 1938, **17:** 392–409.

ROTHBALLER, A. B.: Changes in the rat neurohypophysis induced by painful stimuli with particular reference to neurosecretory material. Anat. Rec., 1953, **115:** 21–41.

SAWYER, C. H., MARKEE, J. E. AND EVERETT, J. W.: Further experiments on blocking pituitary activation in the rabbit and the rat. J. Exp. Zool., 1950, **113:** 659–682.

SLUSHER, M. A. AND ROBERTS, S.: Fractionation of hypothalamic tissue for pituitary-stimulating activity. Endocrinology, 1954, **55:** 245–254.

SPIRTOS, B. N., INGRAM, W. R., BOGDANOVE, E. M. AND HALMI, N. S.: Increased insulin sensitivity in rats with hypothalamic lesions, and its interpretation. J. Clin. Endocrinol., 1954, **14:** 790.

THOMPSON, J. C. AND BLOUNT, R. F.: The age of beginning reactivity of the pituitary-adrenocortical system to stress in the mouse. Endocrinology, 1954, **54:** 620–626.

THOMSON, A. P. D. AND ZUCKERMAN, S.: The effect of pituitary-stalk section on light-induced oestrus in ferrets. Proc. Roy. Soc. London, B., 1954, **142:** 437–451.

VELDEN, R. VON DEN: Die Nierenwirkung von Hypophysenextrakten beim Menschen. Berl. klin. Woch., 1913, **50:** 2083–2086.

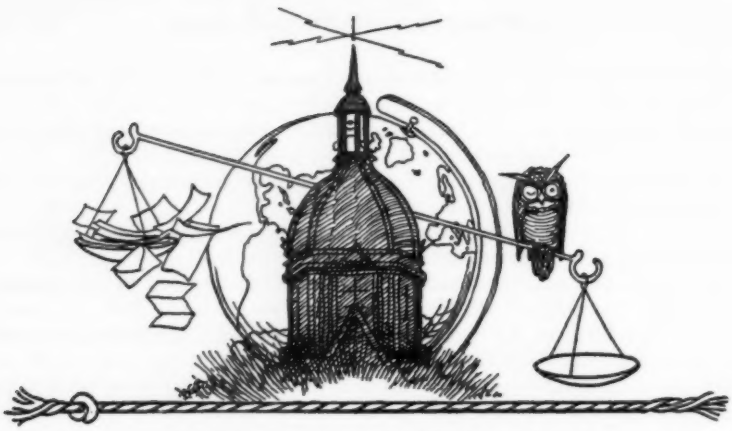
VERNEY, E. B.: The antidiuretic hormone and the factors which determine its release. Proc. Roy. Soc. London, B., 1947, **135:** 25–106.

VOGT, MARTHE: Cortical secretion of the isolated perfused adrenal. J. Physiol., 1951, **113:** 129–156.

WINGSTRAND, K. G.: *The Structure and Development of the Avian Pituitary.* C. W. K. Gleerup, Lund, Sweden, 1951.

WISLOCKI, G. B. AND KING, L. S.: The permeability of the hypophysis and the hypothalamus to vital dyes, with a study of the hypophysial vascular supply. Am. J. Anat., 1936, **58:** 421–472.

XUEREB, G. P., PRICHARD, MARJORIE, M. L., AND DANIEL, P. M.: The hypophysial portal system of vessels in man. Quart. J. Exp. Physiol., 1954, **39:** 219–230.



BOOK REVIEWS

Systemic Lupus Erythematosus. By A. McGehee Harvey, Lawrence E. Shulman, Philip A. Tumulty, C. Lockard Conley and Edyth H. Schoenrich. 437 pp., $3.00. The Williams & Wilkins Co., Baltimore, Md.

This book is a reprint of the authors' recent paper published in *Medicine*. In this work they have analysed 138 carefully studied cases of systemic lupus erythematosus which they have followed in the Johns Hopkins Hospital. After a discussion of the pathology and pathogenesis of the disease, there is a description of the clinical manifestation of lupus organized on the basis of the organ systems involved. The principles of management of systemic lupus are presented together with the effect of treatment on suppressing the various clinical manifestations. In each instance the authors' experience is accompanied by a discussion of the experience of others as obtained from an extensive review of the literature.

R. J. J.

Cellular Metabolism and Infections. New York Academy of Medicine, Section on Microbiology Symposium No. 8. Edited by E. Racker. 196 pp., $4.80. Academic Press, New York, N. Y.

This symposium, held under the auspices of the New York Academy of Medicine, is comparable to its predecessors in being a presentation of stimulating thought and factual data. The symposium is divided into two major parts. One deals with "Aspects of Comparative Biochemistry". In this section the metabolic activities of the infective agent are correlated with those of the host. The biochemical and metabolic similarities and dissimilarities of the host tissues and microorganisms cause a lively discussion on the "biochemical unity of life". This has an important place in the consideration of and experimentation on specific antagonists to disease. The second part of the symposium deals largely with "Metabolic Aspects of the Infectious Process". The specific features of microbial activity which produce infection, and the host's metabolic alteration during infection are discussed at great length.

Much of the information presented represents original work of the authors, all of whom have done outstanding work in the field.

As one reads this symposium it is apparent that there is much to learn about the *how and why* of infectious disease. However, the authors deal effectively with the data avail-

able and indicate the direction in which current investigation may lead to a clearer insight into the problems of cellular metabolism and infection.

LEIGHTON E. CLUFF

Christopher's Minor Surgery, 7th edition. Edited by ALTON OCHSNER and MICHAEL E. DEBAKEY. 547 pp., $9.00. W. B. Saunders Co., Philadelphia, Pa.

Despite the fact that the volume under consideration bears the familiar name of "Christopher's Minor Surgery", few similarities to the older editions are found in the new version edited by Drs. Ochsner and DeBakey. It is essentially a new text rather than a revision of the old.

The present edition is considerably more readable than older editions. The type and paper are good. The change in organization from an anatomic to a system grouping is an improvement. Subject coverage, however, is considerably less extensive and illustrations are fewer. The text has been reduced to about half the number of pages but the actual decrease in text is somewhat greater due to changes in format.

The new version, unlike the old, has been prepared by a number of authors. While certain virtues of this method are obvious, difficulties are also to be noted. Occasional overlap with dual coverage of a subject is found in the new text. Again, omission of certain topics is a potential hazard and it is to be noted that hand infections are not covered in the present volume. Similarly, the sole consideration of the female breast concerns contusions. It may be that the editors do not consider these topics within the realm of "minor surgery", but they seem to the reviewer to be serious omissions in a text directed, at least in part, toward readers at interne and resident level.

The perfect balance between brevity and adequate coverage is difficult to achieve. It is the reviewer's opinion that, while previous editions were perhaps too comprehensive, the pendulum has now swung past the optimum point. This edition should prove highly useful to medical students but will not enjoy the great popularity of the older editions in the sphere in which they found their greatest value; i.e., as a ready reference text for surgeons-in-training.

J. C.

Lehrbuch und Atlas der Anatomie des Menschen, Rauber-Kopsch, 19th edition, in 2 volumes, 1955. By FR. KOPSCH. Volume II: Eingeweide, Nervensystem, Sinnesorgane. 768 pp., Ganzleinen DM 64.50 ($15.35). Georg Thieme Verlag, Stuttgart, Germany.

Volume I of this treatise was the subject of a recent review in this journal (vol. 96, p. 285); the general statements there made are applicable here, and need not be repeated. Again, the volume is abundantly illustrated, and about a third of the figures are in color. The treatise as a whole can be warmly recommended.

A. L. G.

Practical Endocrinology. By LEWIS M. HURXTHAL in cooperation with A. SEYMOUR PARKER and HIRSH SULKOWITCH. 318 pp., $7.00. The Blackiston Division of the McGraw-Hill Book Co., New York, N. Y.

The book is designed to convey to the busy practitioner the basic clinical facts needed for diagnosis and treatment of endocrine disorders. It is well organized, written in clear style and simple terminology. The writer is a skilled clinician, whose therapeutic advice stems from a large experience and sound conservative judgment. There is no doubt that a physician who could assimilate all that this book contains would be in a position to render excellent opinion and advice in the field of endocrinology.

The field of medicine covered in this book is a relatively recent one and research is constantly disclosing new facts and generating new physiological concepts. No two patients, even with the same pathological lesions, present identical clinical symptoms or signs. The practitioner of endocrinology, therefore, needs his own basic knowledge to orient himself in meeting the disease patterns which will so frequently be found to deviate in one or more respects from that of the "pure syndrome". A bibliography would, in the opinion of this reviewer, be most helpful, in order that the reader might at times see for himself the original work from which the author of the book has drawn his conclusions.

JOHN EAGER HOWARD

Stress Situations. Edited by SAMUEL LIEBMAN. 144 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.

This small book contains the Second Annual North Shore Health Resort Lecture Series devoted to "Emotional Reactions to Stress". The material pertains to "the manner in which the personality responds to the pressures of social and personal difficulties", edited essentially as recorded at the time of original presentation.

The lecturers are for the most part distinguished psychoanalysts, and the general tenor of the book is, therefore, expressed in the Freudian sense, with characteristic emphasis on a biologic, behavioristic and material approach. The pursuit of questions to conclusions which could be just as logically propounded in metaphysical terms is avoided. It means that certain issues involved in the study of man's reaction under stress are dealt with in purely physical terms; this limits scope in the analysis of the psyche. Such is especially true in the chapter relative to death and suicide by Jules H. Masserman. One feels a need for a change of pace in thought. A more sensible handling is offered by Leo H. Bartemeier on emotional reactions to marriage, although he leaves a number of practical channels unexplored.

Other topics included in relation to stress are reactions to frustration and failure (Lewis L. Robbins), acute illnesses (Francis J. Gerty), catastrophe (John P. Spiegel), fertility and sterility (Boris B. Rubenstein), and divorce (Harry H. Garner). Each chapter represents a lecture given in an easy, informal style. There is no effort at scholarship and completeness but rather an informative interest-arousal and a highlighting of trends in the sphere discussed. The seven lectures were directed towards the general medical community as well as physicians in the psychiatric residency program, and the book should now serve a similarly wider circle.

EDWARD L. SUAREZ-MURIAS

Analytical Cytology. Newer Methods for Studying Cellular Form and Function. Edited by ROBERT C. MELLORS. Foreword by FRANCIS O. SCHMITT. 512 pp., $15.00. The Blakiston Division, McGraw-Hill Book Co., New York, N. Y.

In the foreword to this book, Professor F. O. Schmitt writes, "It is a thrilling experience to be engaged in research in a great scientific field at a time when that field is just coming into fruition, when new advances seem to be coming along with ever increasing frequency. Such is the situation in that synthesis of cell morphology and biochemistry which has come to be known as analytical cytology and which is ably dealt with in this volume."

Much of the volume is indeed ably written, but the book is not a treatise on cell structure correlated with biochemistry as the title and foreword might suggest. Rather, it is a succession of chapters devoted to optical and chemical methods now finding useful application in investigating the finer structure of cells and tissues and the chemical and physiological significance of the structures encountered. Each chapter is written by a worker

experienced with the method in question—in several instances the authors are outstanding authorities in the fields of which they write. Especially noteworthy are Barer's chapter on various types of interference microscopy, Engströms on historadiography, Pollister's and Ornstein's discussion of microspectroscopy in the visible spectral range and Novikoff's treatment of histochemical and cytochemical staining methods. There are other chapters on electron microscopy, ultraviolet microspectroscopy, fluorescence microscopy, radioautography and X-ray diffraction. Each chapter discusses the principles underlying the method, gives some details of procedure, instrumentation, field of usefulness and limitations, and mentions, usually in a catalog fashion, some of the contributions which have been made to cytology through use of the method. The chapters by Ornstein and Pollister and by Barer contain much that is not readily available in other sources, and which will provide thoughts and points of view of value even to many experienced workers. The remaining chapters may be of use to graduate students or to others seeking a brief introduction to the principles and potentialities of some cytological method. But in these fields an advanced worker will have to look elsewhere for comprehensive treatment of the subject.

One may attempt to view this book with some historical and semantic perspective. The word, "analysis", comes from Greek roots meaning, "to loosen apart." The roots of the word, "anatomy", convey the meaning "to cut apart." Thus these words are almost synonymous in original sense. In usage today, both convey the idea of separating components of a complex system from each other so that each component can be studied more profitably, with the aim of achieving a better understanding of the components, of the whole, and of the interrelationships involved. What, then, is the difference between "analytical cytology" and "anatomical cytology," or any other type of cytology? The term "analytical cytology" has been in use for ten years or so by some who deem it useful to have a word to apply to the integrative approach to cytology—to an approach which seeks to describe and interpret cell morphology and function quantitatively in chemical and molecular terms—to unite the concepts of biochemistry, physiology and morphology at the cellular and molecular levels. The term "analytical cytology" is thus meant to distinguish the integrative and quantitative approach to cytology from other—presumably less esteemed—approaches.

The integrative approach to morphology is indeed laudable, exciting, and flourishing at the present time. It seems evident that the development of the electron microscope and of other technical advances has placed us on the threshold of a new classical period in anatomy—a period comparable in importance to the Vesalian epoch or to the great development of microscopic anatomy ushered in by Bichat and Johannes Müller over a century ago. Yet one must remember that the integrative approach—the heart of the concept of "analytical cytology" is not new. Henle had his approach. So did Kölliker, Bowman, Langley, von Ebner, Miescher, Engelmann, Kühne, Ranvier, Heidenhain, Vlés, Brücke. Conceptually, the writings and works of these great cytologists are integrative. They used every experimental, optical and chemical means at their disposal to explore the chemical and physiological significance of the structures they observed. They brought powerful intellects, broad comprehension of many branches of science, sound critique, and penetrating understanding into their interpretations. Except for the fact that their instrumental and conceptual armamentarium was less powerful than that available to today's workers, their field would fall within the compass of "Analytical Cytology" as conceived by those who value this term. One may well ponder the prescient writings of the great Albert Kölliker, proud to consider himself a histologist and to hold the simple title of Professor of Anatomy and Physiology at Würzburg. In an 1854 translation of the "Historical Introduction" to Kölliker's "Manual of Human Microscopical Anatomy", the first German

edition of which appeared in 1852, the following words can be found; (The italics are Kölliker's) "Microscopical anatomy is concerned with the understanding of the microscopic forms, and with the laws of their structure and development. . . . When the morphological elements have been perfectly made out, the next object is to discover the laws according to which they arise, wherein one must not fail to have regard also to their relations of composition and function. . . . A logical investigation of *the chemical relations of the elementary parts and of their molecular forces*, . . . , combined with a more *profound microscopical examination* of them . . . will assuredly . . . bring us, step by step, nearer to the desired though perhaps never to be reached, end." In another passage, Kölliker says "If it be possible that the molecules which constitute cell-membranes, muscular fibrils, axile fibre of nerves etc., should be discovered, and the laws of their apposition, and of the alteration which they undergo in the course of their origin, the growth, and the activity of the present so-called elementary parts, should be made out, then a new era will commence for Histology and the discoverer of the law of *cell genesis*, or of a *molecular theory*, will be as much or more celebrated than the originator of the doctrine of the composition of all animal tissues out of cells."

It may well be that no single person is to make those great discoveries which Kölliker anticipated, and perhaps even that no single molecular theory or law of cell genesis will achieve overriding importance. Yet it is clear that Kölliker understood clearly the fruitfulness of the integrative and quantitative approach. Many today who wish to make progress toward the goal which Kölliker designated can find aid in the pages of Dr. Mellors' book.

H. Stanley Bennett

Gestation. Transactions of the First Conference March 9, 10 and 11, 1954. Edited by Louis B. Flexner. 238 pp., $5.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

The first Macy Foundation Conference volume on gestation is devoted entirely to the placenta, probably the least respected of all vital organs. Anyone reading this book will gain added respect for the protean nature and functions of this short-lived organ.

The brilliant work of Flexner on placental transport using isotopes is perhaps the most widely known work to be discussed here. The equally important investigations by Huggett and his associates on dextrose and fructose transport in the ungulate placenta are less well known. Unfortunately, the informal conference method of presentation is not well suited to these studies. Such conferences are certainly of great value to the expert participants, but the verbatim reporting of them can only confuse the less well informed. These conferences will certainly provide the philosopher of science with invaluable insight into the functions of the scientific mind, as is promised in the preface. On the other hand, they cannot be as rewarding to the searcher after scientific fact.

The most valuable portion of this book is the discussion centered around the work of Amoroso on the comparative anatomy and histology of the placental barrier. The truly protean nature of the placenta is revealed by the study of its comparative anatomy to which Amoroso has devoted his scientific life. His studies, and those of Wislocki and Dempsey with the electron microscope, have demonstrated the inadequacy of Grosser's classification of placentas according to the number of layers separating the maternal and fetal blood. It is to be hoped that the future Macy Conferences on Gestation will provide us with a more usable classification.

George W. Corner, Jr.

Die Bronchographie. By Ernst Stutz and Heinz Vieten. 250 pp., Ganzleinen DM 66. ($15.70. Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Book Corp., New York, N. Y.

The purpose of this well-published volume is to describe the normal and pathologic anatomy of the bronchial system as visualized on x-ray. There are comprehensive sections on the technique of bronchography, with emphasis on the catheterizing of individual segments.

The text material is a massive compendium of the world-literature on the subject and is sometimes not chosen with an eye for the practical.

The section on normal anatomy and physiology is rich in words, yet poor in clear illustrations. In the section on diseases of the bronchi the illustrations are likewise lacking.

One has the impression that a great wealth of material has been simply gathered and put into book form without sufficiently critical selection and analysis.

D. J. TORRANCE

BOOKS RECEIVED FOR REVIEW

Clinical Biochemistry. By ABRAHAM CANTAROW and MAX TRUMPER. 738 pp., $9.00. W. B. Saunders Co., Philadelphia, Pa.

Clinical Orthopaedics No. 5. Edited by ANTHONY F. DEPALMA. 242 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Counseling in Medical Genetics. By SHELDON C. REED. 268 pp., $4.00. W. B. Saunders Co., Philadelphia, Pa.

Hypotension. Shock and Cardiocirculatory Failure. By PAUL G. WEIL. 78 pp., $2.25. J. B. Lippincott Co., Philadelphia, Pa.

The Josiah Macy, Jr. Foundation 1930–1955. A Review of Activities. 174 pp.

Laboratory Identification of Pathogenic Fungi Simplified. By ELIZABETH L. HAZEN and FRANK CURTIS REED. 108 pp., $5.50. Charles C Thomas, Springfield, Illinois.

Medicine for Nurses, 2nd edition. By M. TOOHEY. With a chapter on Psychological Medicine by HENRY R. ROLLIN. 640 pp., $6.50. The Williams & Wilkins Co., Baltimore, Md. Also E. & S. Livingstone Ltd., Edinburgh and London.

New and Nonofficial Remedies 1955. Issued Under the Direction and Supervision of The Council on Pharmacy and Chemistry, American Medical Association. 653 pp., $3.35. J. B. Lippincott Co., Philadelphia, Pa.

The Pharmacological Basis of Therapeutics, 2nd edition. By LOUIS S. GOODMAN and ALFRED GILMAN. 1831 pp., $17.50. The Macmillan Co., New York, N. Y.

The Principles and Practice of Surgical Nursing. By D. F. ELLISON NASH. 1007 pp., $7.00. The Williams & Wilkins Co., Baltimore, Md. Also Edward Arnold, Publishers, London, England.

Report of the Medical Research Council for the Year 1953–1954. Presented by the Lord President of the Council to Parliament by Command of Her Majesty June 1955. 274 pp., 7s 6d net. Her Majesty's Stationery Office, London, England.

Secretion of Adrenaline and Sympathins. By Y. SATAKE. 158 pp., $4.00. Nanzando Co., Ltd., Hongo, Tokyo, Japan.

Shearer's Manual of Human Dissection, 3rd edition. Edited by CHARLES E. TOBIN. 287 pp., $6.00. McGraw-Hill Book Co., New York, N. Y.

Talking with Patients. By BRIAN BIRD. 154 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.